活動報告

平成１６年度中期－１６年度後期構造生物学坂部プロジェクトの活動

運営委員会委員長
坂 部 知 平

Ⅰ．ＳＢＳＰ用第一実験ステーションＢＬ６Ｂ及びＢＬ６Ｃ

１．利用状況

平成１６年度中期のビームタイムは平成１６年９月２８日（火）午前９時に開始され１２月１９日（日）午前９時に終了した。又、平成１６年度後期のビームタイムは平成１７年１月２０日（木）午前９時に開始され２月２８日（月）午前９時に終了した。平成１６年度中期及び後期のBL6Bの予約状況をそれぞれ表１及び表２に示す。尚ＢＬ６ＣはSuper Galaxy開発のための予備実験として使用したため予約表は用いず利用希望者が坂部知平までメールで連絡する形式をとった。その結果１０月７～９日及び１１月１０～１２日に味の素（株）の柏木立己さん、１２月５日～１２日に中国科学院生物物理研究所のChang Wenrui等のグループ及び、２月２７日～２月２８日に第一製薬の鈴木誠さんが利用された。前回と同様bonus日(入射器のマシンスタディ)を予備日とし、それ以外の予備日は取らなかった。bonus timeは１週間前迄に急を要する要求がなければキャンセルを行った。

２．装置の状況

ビームラインの故障はなかった。低温吹付け装置も正常に稼動した。
Station check sheetから状況を拾うと下記の一件が有ったのみである。
１２月１６日　　コリメーター取付ヶ所付近からＸ線の漏洩あり、鉛箔で止めた。

表１．平成１６年度中期BL6Bビームタイム使用状況

day:	am:9.00-pm:9.00	night: pm9.00-am:9.00(the next day)
9/28 TUE	R bonus_time (day)	bonus_time (night)
9/29 WED	R setting_test (day)	setting_test (night)
9/30 THU	R setting_test (day)	setting_test (night)
10/ 1 FRI	R - (day)	- (night)
10/ 2 SAT	R - (day)	- (night)
10/ 3 SUN	R - (day)	- (night)
10/ 4 MON	R - (day)	- (night)
10/ 5 TUE	R bonus_time (day)	bonus_time (night)
10/ 6 WED	R Daiichi_Pharm._c (day)	- (night)
10/ 7 THU	R Ajinomoto_Co._Inc_c (day)	- (night)
10/ 8 FRI	R Daiichi_Pharm._c (day)	- (night)
10/ 9 SAT	R - (day)	- (night)
10/10 SUN	R - (day)	- (night)
10/11 MON	R machine_study (day)	machine_study (night)

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10/12 TUE R                   - (day)                   - (night)
10/13 WED R  Yamanouchi_Pharm._c (day)                   - (night)
10/14 THU R                   - (day)                   - (night)
10/15 FRI R                   - (day)                   - (night)
10/16 SAT R                   - (day)                   - (night)
10/17 SUN R                   - (day)                   - (night)
10/18 MON R        machine_study (day)       machine_study (night)
10/19 TUE R                   - (day)                   - (night)
10/20 WED R                   - (day)                   - (night)
10/21 THU R                   - (day)                   - (night)
10/22 FRI R                   - (day)                   - (night)
10/23 SAT R                   - (day)                   - (night)
10/24 SUN R                   - (day)                   - (night)
10/25 MON R        machine_study (day)       machine_study (night)
10/26 TUE R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
10/27 WED R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
10/28 THU R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
10/29 FRI R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
10/30 SAT R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
10/31 SUN R    3GeV_single-bunch (day)   3GeV_single-bunch (night)
11/ 1 MON R        machine_study (day)       machine_study (night)
11/ 2 TUE R           bonus_time (day)          bonus_time (night)
11/ 3 WED R               BERI_c (day)                   - (night)
11/ 4 THU R      Chugai_Pharm._c (day)                   - (night)
11/ 5 FRI R      Chugai_Pharm._c (day)                   - (night)
11/ 6 SAT R                   - (day)                   - (night)
11/ 7 SUN R                   - (day)                   - (night)
11/ 8 MON R        machine_study (day)       machine_study (night)
11/ 9 TUE R                   - (day)          bonus_time (night)
11/10 WED R  Yamanouchi_Pharm._c (day)                   - (night)
11/11 THU R  Ajinomoto_Co._Inc_c (day)                   - (night)
11/12 FRI R  Ajinomoto_Co._Inc_c (day)                   - (night)
11/13 SAT R                   - (day)                   - (night)
11/14 SUN R                   - (day)                   - (night)
11/15 MON R        machine_study (day)       machine_study (night)
11/16 TUE R                   - (day)                   - (night)
11/17 WED R                   - (day)                   - (night)
11/18 THU R                   - (day)                   - (night)
11/19 FRI R               BERI_c (day)                   - (night)
11/20 SAT R                   - (day)                   - (night)
11/21 SUN R                   - (day)                   - (night)
11/22 MON R        machine_study (day)       machine_study (night)
11/23 TUE R                   - (day)                   - (night)
11/24 WED R             co_users (day)            co_users (night)
11/25 THU R       Banyu_Pharm._c (day)                   - (night)
11/26 FRI R     overseas_users_a (day)    overseas_users_a (night)
11/27 SAT R     overseas_users_a (day)    overseas_users_a (night)
11/28 SUN R               BERI_c (day)                   - (night)
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11/29	MON	R	machine_study (day)	machine_study (night)
11/30	TUE	R	bonus_time (day)	bonus_time (night)
12/ 1	WED	R	co_users (day)	co_users (night)
12/ 2	THU	R	Yamanouchi_Pharm._c (day)	- (night)
12/ 3	FRI	R	overseas_users_a (day)	overseas_users_a (night)
12/ 4	SAT	R	overseas_users_a (day)	overseas_users_a (night)
12/ 5	SUN	R	- (day)	- (night)
12/ 6	MON	R	machine_study (day)	machine_study (night)
12/ 7	TUE	R	- (day)	- (night)
12/ 8	WED	R	co_users (day)	co_users (night)
12/ 9	THU	R	Ajinomoto_Co._Inc_c (day)	- (night)
12/10	FRI	R	Daiichi_Pharm._c (day)	- (night)
12/11	SAT	R	- (day)	- (night)
12/12	SUN	R	- (day)	- (night)
12/13	MON	R	machine_study (day)	machine_study (night)
12/14	TUE	R	- (day)	- (night)
12/15	WED	R	Kyowa_Hakko_Kogyo_c (day)	- (night)
12/16	THU	R	co_users (day)	co_users (night)
12/17	FRI	R	Banyu_Pharm._c (day)	- (night)
12/18	SAT	R	Daiichi_Pharm._c (day)	- (night)
12/19	SUN	R	- (day)	- (night)

表２．平成１６年度後期 BL6B ビームタイム使用状況

day: am:9.00-pm:9.00　night: pm9.00-am:9.00(the next day)

1/20	THU	R	Camera_maintenance (day)	Camera_maintenance (night)
1/21	FRI	R	Daiichi_Pharm._c (day)	Watanabe_Nobuhisa_d (night)
1/22	SAT	R	- (day)	- (night)
1/23	SUN	R	- (day)	- (night)
1/24	MON	R	machine_study (day)	machine_study (night)
1/25	TUE	R	bonus_time (day)	bonus_time (night)
1/26	WED	R	Yamanouchi_Pharm._c (day)	- (night)
1/27	THU	R	Kyowa_Hakko_Kogyo_c (day)	- (night)
1/28	FRI	R	- (day)	- (night)
1/29	SAT	R	- (day)	- (night)
1/30	SUN	R	- (day)	- (night)
1/31	MON	R	machine_study (day)	machine_study (night)
2/ 1	TUE	R	bonus_time (day)	bonus_time (night)
2/ 2	WED	R	Yamanouchi_Pharm._c (day)	- (night)
2/ 3	THU	R	Ajinomoto_Co._Inc_c (day)	- (night)
2/ 4	FRI	R	- (day)	- (night)
2/ 5	SAT	R	- (day)	- (night)
2/ 6	SUN	R	- (day)	- (night)
2/ 7	MON	R	machine_study (day)	machine_study (night)
2/ 8	TUE	R	3GeV_single-bunch (day)	3GeV_single-bunch (night)
2/ 9	WED	R	3GeV_single-bunch (day)	3GeV_single-bunch (night)
2/10	THU	R	3GeV_single-bunch (day)	3GeV_single-bunch (night)
2/11	FRI	R	3GeV_single-bunch (day)	3GeV_single-bunch (night)
2/12	SAT	R	3GeV_single-bunch (day)	3GeV_single-bunch (night)

2/13 SUN R	3GeV_single-bunch (day)	3GeV_single-bunch (night)
2/14 MON R	machine_study (day)	machine_study (night)
2/15 TUE R	bonus_time (day)	bonus_time (night)
2/16 WED R	Yamanouchi_Pharm._c (day)	- (night)
2/17 THU R	Ajinomoto_Co._Inc_c (day)	- (night)
2/18 FRI R	BERI_c (day)	- (night)
2/19 SAT R	- (day)	- (night)
2/20 SUN R	- (day)	- (night)
2/21 MON R	machine_study (day)	machine_study (night)
2/22 TUE R	co_users (day)	co_users (night)
2/23 WED R	Yamanouchi_Pharm._c (day)	- (night)
2/24 THU R	Kyowa_Hakko_Kogyo_c (day)	- (night)
2/25 FRI R	- (day)	- (night)
2/26 SAT R	- (day)	- (night)
2/27 SUN R	Daiichi_Pharm._c (day)	- (night)
2/28 MON R	- (day)	- (night)

Ⅱ．コンピュータ関係（ネットワークとデータサーバの利用状況）

ネットワークとサーバは大きなトラブルもなく稼働した。/home 領域については１ヶ月以前に作られたファイルは全て消去した。/save 領域については多量に使用しているユーザに email を送り移動/消去を依頼した。今後とも不要なファイルの消去にご協力をお願いしたい。

Ⅲ．委員会報告

１．編集委員会

運営委員会報告参照

Ⅳ．業績紹介

１．Wenrui Chang (National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences)

Crystal structure of spinach major light-harvesting complex at 2.72Å resolution

Nature, **428**, 287-291,(2004)

Zhenfeng Liu[1], Hanchi Yan1, Kebin Wang[2], Tingyun Kuang[2], Jiping Zhang[1], Luiu Gui[1], Xiaomin An[1] and Wenrui Chang[1]

[1]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15th Datun Road, Chaoyang District, Beijing 100101, People's Republic of China

[2]Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, 20th Nanxincun, Xiangshan, Beijing 100093, People's Republic of China

Summary

The major light-harvesting complex of photosystemⅡ (LHC-Ⅱ) serves as the principal solar energy collector in the photosynthesis of green plants and presumably also functions in photoprotection under high-light conditions. Here we report the first X-ray structure of LHC-Ⅱ in icosahedral proteoliposome assembly at atomic detail. One asymmetric unit of a large R32 unit cell contains ten LHC-Ⅱ monomers. The 14 chlorophylls (Chl) in each monomer can be unambiguously distinguished as eight Chla and six Chlb molecules. Assignment of the orientation of the transition dipole moment of each chlorophyll has been achieved. All Chlb are located around the interface between adjacent monomers, and together with Chla they are the basis for efficient light harvesting. Four carotenoid-binding sites per monomer have been observed. The xanthophyll-cycle carotenoid at the monomer-monomer interface may be involved in the non-radiative dissipation of excessive energy, one of the photoprotective strategies that have evolved in plants.

2. Wenrui-Chang（National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences）

pH-profile crystal structure studies of C-terminal despentapeptide nitrite reductase from *Achromobacter cycloclastes*

BBRC, **316**, 107-113, (2004)

Hai-Tao Li[1], Chao Wang[1], Tschining Chang[2], Wen-Chang Chang[2,3], Ming-Yih Liu[4], Jean Le Gall[4], Lu-lu Gui[1], Ji-Ping Zhang[1], Xiao-Min An[1] and Wen-Rui Chang[1]
[1]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15th Datun Road, Chaoyang District, Beijing 100101, China
[2]Institute of Biological Chemistry, Academia Sinica, Taipei 11529, Taiwan
[3]Institute of Biochemical Sciences, National Taiwan University, Taipei 106, Taiwan
[4]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602-7229, USA

Summary
Crystal structures of C-terminal despentapeptide nitrite reductase (NiRc-5) from *Achromobacter cycloclastes* were determined from 1.9 to 2.3Å at pH 5.0, 5.4, and 6.2. NiRc-5, that has lost about 30% activity, is found to possess quite similar trimeric structures as the native enzyme. Electron density and copper content measurements indicate that the activity loss is not caused by the release of type 2 copper (T2Cu) pH-profile structural comparisons with native enzyme reveal that the T2Cu active center in NiRc-5 is perturbed, accounting for the partial loss of enzyme activity. This perturbation likely results from the less constrained conformations of two catalytic residues. Asp98 and His255. Hydrogen bonding analysis shows that the deletion of five residues causes a loss of more than half the intersubunit hydrogen bonds mediated by C-terminal tail. This study shows that the C-terminal tail plays an important role in controlling the conformations around the T2Cu site at the subunit interface, and helps keep the optimum microenvironment of active center for the full enzyme activity of AcNiR.

3. 鎌田　健司（万有製薬）

Structural Basis for Allosteric Regulation of the Monomeric Allosteric Enzyme Human Glucokinase

Structure, **12,** 429-438**, (**2004**)**

Kenji Kamata, Morihiro Mitsuya, Teruyuki Nishimura, Jun-ichi Eiki and Yasufumi Nagata
Banyu Tsukuba Research Institute in collaboration with Merck Research Laboratories. Okubo 3, Tsukuba, lbaraki 300-2611, Japan

Summary
Glucokinase is a monomeric enzyme that displays a low affinity for glucose and a sigmoidal saturation curve for its substrate, two properties that are important for its playing the role of a glucose sensor in pancreas and liver. The molecular basis for these two properties is not well understood. Herein we report the crystal structures of glucokinase in its active and inactive forms, which demonstrate that global conformational change, including domain reorganization, is induced by glucose binding. This suggests that the positive cooperativity of monomeric glucokinase obeys the “mnemonical mechanism” rather than the well-known concerted model. These structures also revealed an allosteric site through which small molecules may modulate the kinetic properties of the enzyme. This finding provided the mechanistic basis for activation of glucokinase as a potential therapeutic approach for treating type 2 diabetes mellitus.

4.　三木　邦夫（京都大学）、　深海　隆明（京都大学、現在中外製薬）
Crystal Structure of Chaperonin-60 from *Paracocus denitrificans*

J. Mol.Biol. **312**, 501-509, (2001)

Takaaki A. Fukami[1], Masafumi Yohda[2], Hideki Taguchi[3], Masasuke Yoshida[3] and Kunio Miki[1,4]
[1]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[2]Department of Biotechnology and Life Science, Tokyo University of agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo, 184-8588 Japan
[3]Chemical Resources Laboratory, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 226-8503 Japan
[4]RIKEN Harima Institute/SPring-8, 1-1-1 Koto, Mikazuki-cyo, Sayo-gun, Hyogo, 679-5148 Japan

Summary
The crystal structure of chaperonin-60 from *Paracoccus denitrificans* (P.cpn60) has been determined at 3.2Å resolution by the molecular replacement method. Two heptameric rings of identical subunits of P.cpn60 in adjacent asymmetric units are stacked in a back-to-back manner and form a cylinder, as found in GroEL, cpn60 from *Escherichia coli*. With respect to the unliganded GroEL structure, each subunit of P.cpn60 tilts 2˚ outwards and the apical domain twists 4˚ counter-clockwise in the top view in a hinge-like manner, rendering the central hole 5Å wider. Despite the subunit tilts, both rings in P.cpn60 contact at two sites of the equatorial domain in the same way as in GroEL. Interactions between residues 434 and 434, and 463 and 463 observed in GroEL were not found in P.cpn60, and the interaction between 452 and 461 was weaker in P.cpn60 than in GroEL. The unique hydrogen bond between 468 and 471 was

observed at the right site in P.cpn60, which could account for why the subunits tilt outwards. The contact surface area was reduced at the left site, which is similar to the observed changes in the GroEL structures induced by ATP binding. In general, inter-ring interactions in P.cpn60 were weakened, which is consistent with findings that P.cpn60 is observed in single-ring forms as well as in double-ring forms.

5. 三木　邦夫（京都大学）

Crystal Structure of a Novel-Type Archaeal Rubisco with Pentagonal Symmetry

Structure **9** 473-481, **(**2001)

Ken Kitano[1], Norihiro Maeda[2], Toshiaki Fukui[2], Haruyuki Atomi[2], Tadayuki lmanaka[2] and Kunio Miki[1,3]
[1]Department of Chemistry, Graduate School of Science, Kyoto University
Sakyo-ku, Kyoto, 606-8502 Japan
[2]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Sakyo-ku, Kyoto, 606-8501 Japan
[3]RIKEN Harima Institute/SPring-8, Koto, Mikazukicho, Sayo-gun, Hyogo, 679-5148 Japan

Summary
Background: Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the key enzy me of the Calvin-Benson cycle and catalyzes the primary reaction of CO2 fixation in pl ants, algae, and bacteria. Rubiscos have been so far classified into two types. Type I is composed of eight large subunits (L subunits) and eight small subunits (S subunits) with tetragonal symmetry (L8S8), but typeⅡ is usually composed only of two L subunits (L 2). Recently, some genuinely active Rubiscos of unknown physiological function have be en reported from archaea.

Results: The crytal structure of Rubisco from the hyperthermophilic archaeon Thermoco ccus kodakaraensis KOD1(Tk-Rubisco) was determined at 2.8Å resolution. The enzyme is composed only of L subunits and showed a novel (L2)5 decameric structure. Compared to previously known type I enzymes, each L2 dimer is inclined approximately 16º to f orm a toroid-shaped decamer with its unique L2-L2 interfaces. Differential scanning calo rimetry (DSC), circular dichroism (CD), and gel permeation chromatography (GPC) show ed that Tk-Rubisco maintains its secondary structure and decameric assembly even at hig h temperatures.

Conclusions: The present study provides the first structure of an archaeal Rubisco, an u nprecedented(L2)5 decamer. Biochemical studies indicate that Tk-Rubisco maintains its de cameric structure at high temperatures. The structure is distinct from type I and typeⅡR ubiscos and strongly supports that Tk-Rubisco should be classified as a novel typeⅢ Ru bisco.

6. 祥雲　弘文（東京大学）、伏信　進矢（東京大学）

Chitobiose Phosphorylase from *Vibrio proteolyticus*, a Member of Glycosyl Transferase Family 36, Has a Clan GH-L-like $(\alpha/\alpha)_6$ Barrel Fold

Structure, **12**, 937-947, (June 2004

Masafumi Hidaka[1], Yuji Honda[2], Motomitsu Kitaoka[2], Satoru Nirasawa[2], Kiyoshi Hayashi[2], Takayoshi Wakagi[1], Hirofumi Shoun[1] and Shinya Fushinobu[1]
[1]Department of Biotechnology, The University of Tokyo 1-1-1, Yayoi, Bunkyo-ku, Tokyo, 113-8657 Japan
[2]National Food Research Institute, 2-l-12, Kannondai, Tsukuba, lbaraki, 305-8642 Japan

Summary
Vibrio proteolyticus chitobiose phosphorylase (ChBP) belongs to glycosyl transferase family 36 (GT-36), and catalyzes the reversible phosphorolysis of chitobiose into α-GlcNAc-1-phosphate and GlcNAc with inversion of the anomeric configuration. As the first known structures of a GT-36 enzyme, we determined the crystal structure of ChBP in a ternary complex with GlcNAc and SO_4. It is also the first structures of an inverting phosphorolytic enzyme in a complex with a sugar and a sulfate ion, and reveals a pseudo-ternary complex structure of enzyme-sugar-phosphate. ChBP comprises a β sandwich domain and an $(\alpha/\alpha)_6$ barrel domain, constituting a distinctive structure among GT families. Instead, it shows significant structural similarity with glycoside hydrolase (GH) enzymes, glucoamylases (GH-15), and maltose phosphorylase (GH-65) in clan GH-L. The structural similarity reported here, together with distant sequence similarities between ChBP and GHs, led to the reclassification of family GT-36 into a novel GH family, namely GH-94.

Conclusions: The present study provides the first structure of an archaeal Rubisco, an u nprecedented(L2)5 decamer. Biochemical studies indicate that Tk-Rubisco maintains its de cameric structure at high temperatures. The structure is distinct from type Ⅰ and typeⅡ Rubiscos and strongly supports that Tk-Rubisco should be classified as a novel typeⅢ Rubisco.

7.　伏信　進矢（東京大学）

Crystal Structure of Non-Allosteric L-Lactate Dehydrogenase From *Lactobacillus pentosus* at 2.3Å Resolution: Specific Interactions at Subunit Interfaces

Proteins, **46**, 206-214, (2002)

Hiroyuki Uchikoba[1], Shinya Fushinobu[1], Takayoshi Wakagi[1], Michiko Konno[2], Hayao Taguchi[3] and Hiroshi Matsuzawa[4]
[1]Department of Biotechnology, The University of Tokyo, Tokyo, Japan
[2]Department of Chemistry, Faculty of Science, Ochanomizu University, Tokyo, Japan
[3]Department of Applied Biological Science, Science University of Tokyo, Chiba, Japan
[4]Department of Bioscience and Biotechnology, Aomori University, Aomori, Japan

Summary
L-Lactate dehydrogenase (LDH) from *Lactobacillus pentosus* is a non-allosteric enzyme, which

shows, however, high sequence similarity to allosteric LDHs from certain bacteria. To elucidate the structural basis of the absence of allostery of *L. pentosus* LDH (LPLDH), we determined the crystal structure of LPLDH at 2.3Å resolution. Bacterial LDHs are tetrameric enzymes composed of identical subunits and exhibit 222 symmetry. The quaternary structure of LPLDH was similar to the active conformation of allosteric LDHs. Structural analysis revealed that the subunit interfaces of LPLDH are optimized mainly through hydrophilic interactions rather than hydrophobic interactions, compared with other LDHs. The subunit interfaces of LPLDH are more specifically stabilized by increased numbers of intersubunit salt bridges and hydrogen bonds, and higher geometrical complementarity. Such high specificity at the subunit interfaces should hinder the rearrangement of the quaternary structure needed for allosteric regulation and thus explain the "non-allostery" of LPLDH.

8. 祥雲　弘文（東京大学）、伏信　進矢（東京大学）

Chitobiose Phosphorylase from Vibrio proteolyticus, a Member of Glycosyl Transfera se Family 36, Has a Clan GH-L-like (α/α)6 Barrel Fold

Structure, **12,** 937-947, (June 2004)

Masafumi Hidaka[1], Yuji Honda[2], Motomitsu Kitaoka[2], Satoru Nirasawa[2],Kiyoshi Hayashi[2], Takayoshi Wakagi[1], Hirofumi Shoun[1] and Shinya Fushinobu[1]
[1]Department of Biotechnology, The University of Tokyo 1-1-1, Yayoi, Bunkyo-ku, Tokyo, 113-8657 Japan
[2]National Food Research Institute, 2-l-12, Kannondai, Tsukuba, lbaraki, 305-8642 Japan

Summary
Vibrio proteolyticus chitobiose phosphorylase (ChBP) belongs to glycosyl transferase fami ly 36 (GT-36), and catalyzes the reversible phosphorolysis of chitobiose into α-GlcNAc-1 -phosphate and GlcNAc with inversion of the anomeric configuration. As the first known structures of a GT-36 enzyme, we determined the crystal structure of ChBP in a ternary complex with GlcNAc and SO4. It is also the first structures of an inverting phosphorol ytic enzyme in a complex with a sugar and a sulfate ion, and reveals a pseudo-ternary complex structure of enzyme-sugar-phosphate. ChBP comprises a β sandwich domain and an (α/α)6 barrel domain, constituting a distinctive structure among GT families. Instead, it shows significant structural similarity with glycoside hydrolase (GH) enzymes, glucoam ylases (GH-15), and maltose phosphorylase (GH-65) in clan GH-L. The structural similar ity reported here, together with distant sequence similarities between ChBP and GHs, led to the reclassification of family GT-36 into a novel GH family, namely GH-94.

9. 木下　誉富（藤沢薬品工業）、割鞘　雅一（藤沢薬品工業）

Cloning, expression, purification, crystallization and preliminary diffraction analysis of the C-terminal catalytic domain of human poly(ADP-ribose) polymerase

Acta Cryst., **D60**, 109-111, (2004)

Takayoshi Kinoshita, Takeshi Tsutsumi, Riyo Maruki, Masaichi Warizaya, Yoshinori Ishii and

Takashi Fujii
Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd,　5-2-3 Tokodai, Tsukuba, lbaraki 300-2698, Japan

Summary
Two fragments of the C-terminal catalytic domain of human poly(ADP-ribose) polymerase (catPARP), Met-catPARP and Gly-Ser-catPARP, were purified and crystallized. Both catPARP crystals belong to space group C2, with almost the same unit-cell parameters. However, the shapes and harvest periods of both crystals were quite different owing to the slight mutation at the N-terminal position. Gly-Ser-catPARP was found to be more suitable for X-ray crystallography and crystals showed diffraction to at least 3.5 Å resolution.

10. 木下　誉富（藤沢薬品工業)、割鞘　雅一（藤沢薬品工業）
Inhibitor-induced structural change of the active site of human poly(ADP-ribose) polymerase

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Takayoshi Kinoshita[1], Isao Nakanishi[1], Masaichi Warizaya[1], Akinori Iwashita[2], Yoshiyuki Kido[3], Kouji Hattori[3] and Takashi Fujii[1]
[1]Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd. 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan
[2]Medicinal Biology, Research Laboratories, Fujisawa Pharmaceutical Co. Ltd. Kashima 2-l-6, Yodogawa-ku. Osaka 532-8514, Japan
[3]Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co. Ltd. Kashima 2-l-6, Yodogawa-ku, Osaka 532-8514, Japan

Summary
The crystal structure of human recombinant poly(ADP-ribose) polymerase (PARP) complexed with a potent inhibitor, FR257517, was solved at 3.0Å resolution. The fluorophenyl part of the inhibitor induces an amazing conformational change in the active site of PARP by motion of the side chain of the amino acid, Arg878, which forms the bottom of the active site. Consequently, a corn-shaped hydrophobic subsite, which consists of the side chains of Leu769, Ile879, Pro881, and the methylene chain of Arg878, newly emerges from the well-known active site.

11. 祥雲　弘文・伏信　進矢(東京大学)
Structural Evidence for Direct Hydride Transfer from NADH to Cytochrome P450nor

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Reiko Oshima[1], Shinya Fushinobu[1], Fei Su[2], Li Zhang[2], Naoki Takaya[2] and Hirofumi Shoun[1]

[1]Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113-8657, Japan
[2]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Summary
Nitric oxide reductase cytochrome P450nor catalyzes an unusual reaction, direct electron transfer from NAD(P)H to bound heme. Here, we succeeded in determining the crystal structure of P450nor in a complex with an NADH analogue, nicotinic acid adenine dinucleotide, which provides conclusive evidence for the mechanism of the unprecedented electron transfer. Comparison of the structure with those of dinucleotide-free forms revealed a global conformational change accompanied by intriguing local movements caused by the binding of the pyridine nucleotide. Arg64 and Arg174 fix the pyrophosphate moiety upon the dinucleotide binding. Stereo-selective hydride transfer from NADH to NO-bound heme was suggested from the structure, the nicotinic acid ring being fixed near the heme by the conserved Thr residue in the I-helix and the upward-shifted propionate side-chain of the heme. A proton channel near the NADH channel is formed upon the dinucleotide binding, which should direct continuous transfer of the hydride and proton. A salt-bridge network (Glu71-Arg64-Asp88) was shown to be crucial for a high catalytic turnover.