構造生物　Vol.６ No２
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活動報告

平成１２年度前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．ＴＡＲＡ用第１実験ステーションＢＬ６Ｂ

１．利用状況

平成１２年度前期のビームタイムは平成１２年４月６日（木）から開始され７月２５日（火）に終了した（表１）。前回と同様bonus日(入射器のマシンスタディー)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。

例年７月のビームタイムは８～１０日に終了していたが、今年は２３日まであったので、可成りの余裕があった。

２．ビームラインアシスタント

今期は殆ど各期間一人でお願いした。
今期のビームラインアシスタント名簿を掲載すると共に諸氏に感謝する。

氏　　名	所　　属	期　　間
水野　伸宏	京都大学・樋口・三木研	4月 6日～ 4月29日
西條　慎也	東京工業大学・田中研	5月 8日～ 6月 1日
日下部吉男	昭和大学. 中村研	5 月29日～6月27日
中嶋　義隆	大阪市立大学・広津研	6月30日～ 7月25日

３．装置の状況

今期station checking sheetに書かれたコメントは合計１３件あった（表２）。今期は長かったにも拘わらず故障の報告は極めて少なかった。先回最も多かったClose Doorは１回のみであった。中丸幸雄氏による調整が功を奏したものと思われる。今回最も多かったのはＩＰ２の真空エラーでこれが３回記録されていた。シャットダウン中に修理する。記録中最も気になるのは７月１６日にＩＰ５で生じた「読み取った画像に横に筋が観測された」ことであったが、中丸幸雄氏に問い合わせたところ、既にレベル調整で修復したとのことである。

この他記録には無かったが、試料結晶の回転軸方向の動き（Ｚ方向）を緩く設定するとωの動きに伴ってずれを生ずることがあった。硬く設定すると移動させるのに力がいる。これに対する改良が８月１１日に理学の横沢氏によって行われた。

表１．平成１２年度前期ＢＬ６Ｂビームタイム使用状況

Schedule of BL6B 2000 (1)

LAST UPDATE: 6/18/2000 14:27
USER NAME _a:administer _c:industry _d:non-industry -:free
A:50% area for long works, B:50% area for short works
day: am:9.00-pm:9.00 night: pm9.00-am:9.00(the next day)

4/ 6	THU	B	machine_study	(day)	machine_study (night)
4/ 7	FRI	B	-	(day)	- (night)
4/ 8	SAT	B	-	(day)	- (night)
4/ 9	SUN	B	-	(day)	- (night)
4/10	MON	B	machine_study	(day)	machine_study (night)
4/11	TUE	B	bonus_time	(day)	- (night)
4/12	WED	B	Yamanouchi_Pharm._c	(day)	- (night)
4/13	THU	B	Ajinomoto_Co._Inc_c	(day)	Ajinomoto_Co._Inc_c (night)
4/14	FRI	B	Banyu_Pharm._c	(day)	- (night)
4/15	SAT	B	JT_c	(day)	JT_c (night)
4/16	SUN	B	Sankyo_Co._Ltd_c	(day)	Sankyo_Co._Ltd_c (night)
4/17	MON	A	machine_study	(day)	machine_study (night)
4/18	TUE	A	Fujisawa_Pharm._c	(day)	- (night)
4/19	WED	A	Banyu_Pharm._c	(day)	- (night)
4/20	THU	A	-	(day)	- (night)
4/21	FRI	A	Banyu_Pharm._c	(day)	- (night)
4/22	SAT	A	-	(day)	- (night)
4/23	SUN	A	-	(day)	- (night)
4/24	MON	A	-	(day)	- (night)
4/25	TUE	A	Yamanouchi_Pharm._c	(day)	Tanaka_Isao_d (night)
4/26	WED	A	-	(day)	- (night)
4/27	THU	A	Ajinomoto_Co._Inc_c	(day)	Ajinomoto_Co._Inc_c (night)
4/28	FRI	A	NO_beam	(day)	NO_beam (night)
4/29	SAT	A	NO_beam	(day)	NO_beam (night)
4/30	SUN	A	NO_beam	(day)	NO_beam (night)
5/ 1	MON	A	NO_beam	(day)	NO_beam (night)
5/ 2	TUE	A	NO_beam	(day)	NO_beam (night)
5/ 3	WED	A	NO_beam	(day)	NO_beam (night)
5/ 4	THU	A	NO_beam	(day)	NO_beam (night)
5/ 5	FRI	A	NO_beam	(day)	NO_beam (night)
5/ 6	SAT	A	NO_beam	(day)	NO_beam (night)
5/ 7	SUN	A	NO_beam	(day)	NO_beam (night)
5/ 8	MON	A	machine_study	(day)	machine_study (night)
5/ 9	TUE	A	machine_study	(day)	machine_study (night)
5/10	WED	A	beam_maintenance	(day)	- (night)
5/11	THU	A	-	(day)	Tanaka_Isao_d (night)
5/12	FRI	A	-	(day)	- (night)
5/13	SAT	A	-	(day)	- (night)
5/14	SUN	A	-	(day)	- (night)
5/15	MON	B	machine_study	(day)	machine_study (night)
5/16	TUE	B	-	(day)	- (night)
5/17	WED	B	-	(day)	- (night)
5/18	THU	B	-	(day)	- (night)
5/19	FRI	B	-	(day)	- (night)
5/20	SAT	B	-	(day)	- (night)
5/21	SUN	B	-	(day)	- (night)
5/22	MON	A	machine_study	(day)	machine_study (night)
5/23	TUE	A	bonus_time	(day)	bonus_time (night)
5/24	WED	A	Eisai_Co._Ltd_c	(day)	- (night)
5/25	THU	A	Yamanouchi_Pharm._c	(day)	- (night)
5/26	FRI	A	Daiichi_Pharm._c	(day)	- (night)
5/27	SAT	A	-	(day)	- (night)
5/28	SUN	A	-	(day)	- (night)
5/29	MON	B	machine_study	(day)	machine_study (night)

5/30	TUE	B	-	(day)	- (night)
5/31	WED	B	Banyu_Pharm._c	(day)	- (night)
6/ 1	THU	B	beam_maintenance	(day)	beam_maintenance (night)
6/ 2	FRI	B	beam_maintenance	(day)	beam_maintenance (night)
6/ 3	SAT	B	beam_maintenance	(day)	beam_maintenance (night)
6/ 4	SUN	B	beam_maintenance	(day)	beam_maintenance (night)
6/ 5	MON	A	machine_study	(day)	machine_study (night)
6/ 6	TUE	A	-	(day)	- (night)
6/ 7	WED	A	Fujisawa_Pharm._c	(day)	- (night)
6/ 8	THU	A	-	(day)	- (night)
6/ 9	FRI	A	-	(day)	- (night)
6/10	SAT	A	-	(day)	- (night)
6/11	SUN	A	-	(day)	- (night)
6/12	MON	B	machine_study	(day)	machine_study (night)
6/13	TUE	B	-	(day)	- (night)
6/14	WED	B	Eisai_Co._Ltd_c	(day)	- (night)
6/15	THU	B	Banyu_Pharm._c	(day)	- (night)
6/16	FRI	B	Yamanouchi_Pharm._c	(day)	- (night)
6/17	SAT	B	-	(day)	- (night)
6/18	SUN	B	-	(day)	- (night)
6/19	MON	A	machine_study	(day)	machine_study (night)
6/20	TUE	A	-	(day)	- (night)
6/21	WED	A	-	(day)	- (night)
6/22	THU	A	Mitsubishi_Chem._c	(day)	- (night)
6/23	FRI	A	Eisai_Co._Ltd_c	(day)	- (night)
6/24	SAT	A	-	(day)	- (night)
6/25	SUN	A	-	(day)	- (night)
6/26	MON	A	machine_study	(day)	machine_study (night)
6/27	TUE	A	-	(day)	- (night)
6/28	WED	A	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
6/29	THU	A	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
6/30	FRI	A	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
7/ 1	SAT	A	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
7/ 2	SUN	A	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
7/ 3	MON	B	machine_study	(day)	machine_study (night)
7/ 4	TUE	B	Yamanouchi_Pharm._c	(day)	- (night)
7/ 5	WED	B	-	(day)	Nippon_Roche_c (night)
7/ 6	THU	B	Kyowa_Hakko_Kogyo_c	(day)	Kyowa_Hakko_Kogyo_c (night)
7/ 7	FRI	B	Banyu_Pharm._c	(day)	- (night)
7/ 8	SAT	B	-	(day)	- (night)
7/ 9	SUN	B	-	(day)	- (night)
7/10	MON	A	machine_study	(day)	machine_study (night)
7/11	TUE	A	Eom_Soo_Hyun_b	(day)	- (night)
7/12	WED	A	Banyu_Pharm._c	(day)	- (night)
7/13	THU	A	-	(day)	Nakagawa_Atsushi_d (night)
7/14	FRI	A	Fujisawa_Pharm._c	(day)	Nakagawa_Atsushi_d (night)
7/15	SAT	A	Yamanouchi_Pharm._c	(day)	Takenaka_Akio_d (night)
7/16	SUN	A	Tsukihara_Tomitake_d	(day)	Tsukihara_Tomitake_d (night)
7/17	MON	B	machine_study	(day)	machine_study (night)
7/18	TUE	B	-	(day)	Hirotsu_Ken_d (night)
7/19	WED	B	Mitsubishi_Chem._c	(day)	Mitsubishi_Chem._c (night)
7/20	THU	B	Banyu_Pharm._c	(day)	Se_Won_Suh_b (night)
7/21	FRI	B	Nippon_Roche_c	(day)	- (night)
7/22	SAT	B	Se_Won_Suh_b	(day)	Se_Won_Suh_b (night)
7/23	SUN	B	overseas_users_a	(day)	- (night)
7/24	MON	A	machine_study	(day)	machine_study (night)
7/25	TUE	A	NO_beam	(day)	NO_beam (night)

表２　今期発生した装置（主に大型読取装置）の問題点

月　日	装置名	エラーの種類
４月１２日	カメラ	ゴニオメーターに緩みあり
	ＩＰ６	Doorの締まりが悪い（close Door)
１７日	ＩＰ６	排出エラー
５月２４日	ＩＰ２	ジャモリ
２６日	ＩＰ２	真空エラー
６月　１日	ＩＰ５	ＲＳ２３２Ｃエラー
１６日	ＩＰ５	ドラム回転エラー
７月　４日	ＩＰ２	真空エラー
１４日	ＩＰ２	真空エラー
１６日	ＩＰ５	読み取った画像に横に筋が観測された
１９日	ＩＰ５	バックポジションセンサーエラー
		挿入エラー
		ＲＳ－２３２Ｃエラー

Ⅱ．ＴＡＲＡ第２実験ステーションＢＬ６Ｃ

毎回述べているように、ＢＬ６Ｃには日本学術振興会未来開拓推進事業の産学連携研究費で開発中の全自動データ収集システム（愛称；galaxy）が設置されている。早くオープンせよと言う圧力は大きいが、もう少し待って頂きたい。

１．ＧＵＩによる操作

此まではコマンドによりGalaxyを操作していたが､今回ＧＵＩによる操作が可能になった。此により、手軽に操作できるようになったので、多くの繰り返し運転を行い問題点を探し出した。また誤操作により動作不能になっては困るので、多くの誤操作テストも行った。此らのテストは処置を行う必要があり大変時間の掛かることである。今後も続ける必要がある。

ＧＵＩによる操作が可能になったので、７月１２日行事委員会主催の講習会を開催し、Galaxyに関する活発な意見を頂いた。詳細は次号に掲載する。頂いた意見を基に出来るだけ使い易い装置にしたい。

２．搬送系

正常な操作により概ね、順調に作動するようになったが、時折消去部からカセットが搬送台に送られて来た所で止まってしまう現象が現れた。ハード的にはセンサーは正常に働くがソフト的には異常を見付けて止まってしまう。原因がなかなか見付からず困ったが、結局、「動作終了時点でセンサーは正常に作動しハードは正常値（ＯＮ）を示すが、その後カセットがバンパーにより少し後ずさりしてしまいセンサーがＯＦＦになったとき、ソフトが安全確認のためセンサーの信号を見に行くとＯＦＦなので止まってしまう」ことが分かった。分かってみると笑い話であるが、分かるまで悲痛であった。この他細かい調整が行われた。搬送は結構難しいもので今後多くの改良点が見付かってくるものと思われる。

２．カメラ部

正常に作動するが、改良すべき点も残っている。此までのものよりヘリウムチェンバーの体積が５倍ほど大きいためヘリウムが大量に必要である。密閉性を良くするよう改良を行う。

リゾチーム結晶を用い、データー収集のテストを行ったところビームストッパー周りに激しい、バックグランドノイズが現れた。X線がビームストッパーから漏れたためと判断しその処置を行ったが、ノイズは半減しただけであった。色々なテストを行った結果、バックスキャタリングであることが判明した。即ち、最も大きな原因はコリメータの第一スリットで上流方向に反射したX線がＩＰにバックグランドノイズを与えるのである。これは完全円筒形のＩＰカセットを用いているためで、通常のカメラでは全く観測されないものである。本年度中にＩＰカセットの上流に４象限スリットを取り付ける計画である。これが第一スリットの役をするので、コリメーターの第一スリットを外しテストを行ったところ、バックスキャッタリングは可成り減ったが、未だ有害なほどに残っている。これは空気散乱に依るものと推定される。これはスクリーンとＩＰカセットの間に存在する１０mmのギャップをスリット状の金具を用い危険の無い程度に縮めるなどして改良する予定である。

３．読取部

チェッキングソースで露光したＩＰカセットを読取部に入れmisc中のIP-readを実行したところ動かなかったので、stopしdata collect 中のIP-readを実行したところ轟音がしたのでresetで止めたが、既に動力をＩＰカセットに伝えるカップリングが破壊されていた。
原因を究明中である。バグがどこかにあるはずである。ＩＰカセットを工場に送り９月中に修理する予定である。

４．消去部

ナトリウムランプによる消去は強力であるが、紫外線を等により出来たカラーセンターには蛍光灯の方が良いと言う、フジフイルムの秋元氏の意見に従い、１本置きに３０Wの蛍光灯と入れ替えた。

Ⅲ．コンピュータ関係

１．ネットワークとデータサーバの利用状況

BL6A/BL18BではCCDユーザが多くなり、データサーバの利用が比較的少なくなったため、データ保存期間を７日間に固定して運用することができた。サーバーに書き込まれたデータの総量／日を図１に示す。

２．予告なしの停電

５月１４日の１７時３０分頃、瞬間停電がありデータサーバが止まったが、利用者はBL18BでのCCDユーザのみだったので影響はなかった。

３．InsightⅡおよび QUANTA の設定

上記のMSIのソフトウェアに関してFloating license 契約に従い、SGIのコンピュータへのモジュールの再設定がされた。

４．構造生物学坂部プロジェクトのメーリングリスト

sbsp@sbsp.kek.jpへメールを送るとすべてのプロジェクトメンバーに同じメールが送られるメーリングリストを立ち上げた。

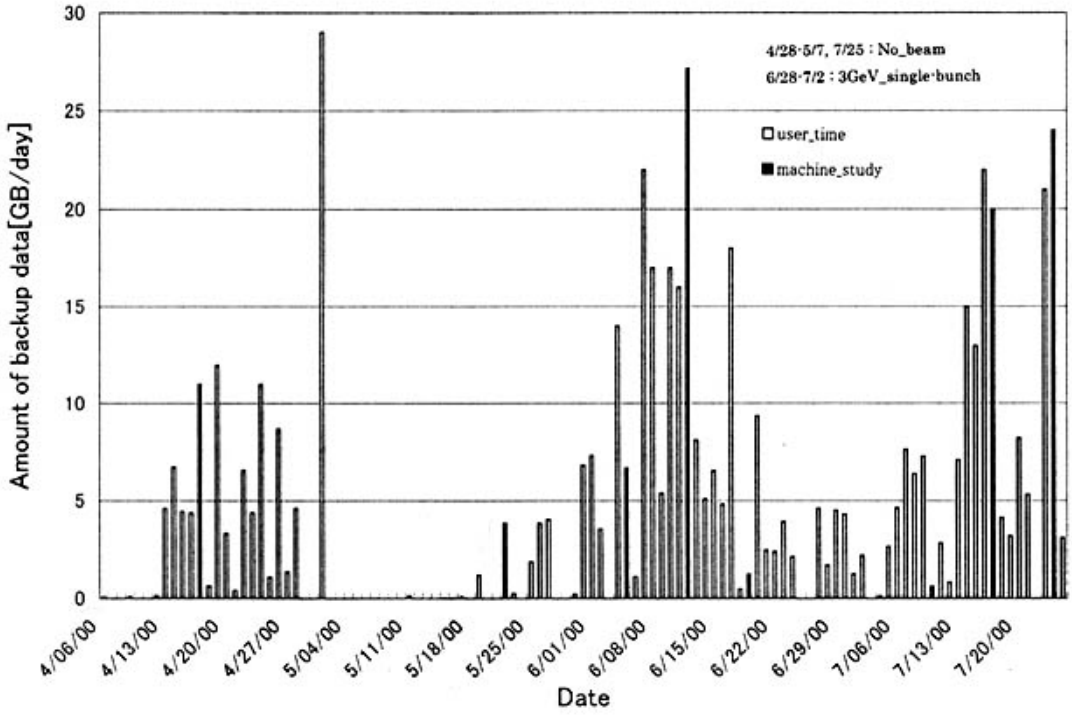

図１．平成１１年後期に於けるＤＬＴバックアップ状況

Ⅳ．各種委員会報告

１．編集委員会

第１５回編集委員会が平成１２年７月１４日開催された。出席者は、石川弘紀、栗原宏之、坂部貴和子、坂部知平、曽我部智、田仲可昌（五十音順）の６名であった。構造生物 Vol.6, No.2 の原稿最終チェックならびに印刷等のスケジュール確認が行われた。続いて、次号(Vol.6, No.3)の内容についての検討が行われ、執筆をお願いする方々を決定した。

２．行事委員会報告

平成１２年７月１２日（水）１０：３０～１２：００に KEK の PF 内光源棟実験ホール BL6C 前にて、坂部知平氏とマックサイエンス社の井上氏による「第一回 BL6C 実験ステーション使用説明会」を開催した。自動 X 線回折測定装置 Galaxy の起動から始まり、データ収集に至る迄の全操作の説明があった。より良い装置を目指して、講師と参加者の
間で、質問や要望などが熱心に行われた。参加者は３５名である。

また、同日、KEK の TARA ハウス２階で１３：３０～１６：３０の間、「第３回 MSI 構造生物ソフトウェア説明会」を開催した。MSI 社の生体高分子分野製品開発担当ディレクター Mary Donlan 博士らに、X-PLOR の拡張後継ソフトウェアの CNX を中心に、構造生物と構造・機能ゲノム関連モジュールと応用例について、紹介していただいた。参加者は２８名である。

Ⅴ．業績紹介

１．祥雲弘文（筑波大）

Cytochrome P450nor, a Novel Class of Mitochondrial Cytochrome P450 Involved in Nitrate Respiration in the Fungus *Fusarium oxysporum*

Archives of Biochemistry and Biophysics, **372**, 340-346 (1999)

Naoki Takaya[1], Sawako Suzuki[1], Seigo Kuwazaki[1], Hirofumi Shoun[1, 3], Fumiaki Maruo[2], Masashi Yamaguchi[4] and Kanji Takeo[4]

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[4] Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, Japan

Summary

Fusarium oxysporum, an imperfect filamentous fungus performs nitrate respiration under limited oxygen. In the respiratory system, Cytochrome P450nor (P450nor) is thought to catalyze the last step; reduction of nitric oxide to nitrous oxide. We examined its intracellular localization using enzymatic, spectroscopic, and immunological analyses to show that P450nor is found in both the mitochondria and the cytosol. Translational fusions between the putative mitochondrial targeting signal on the amino terminus of P450nor and *Escherichia coli* β-galactosidase resulted in signifcant β-galactosidase activity in the mitochondrial fraction of nitrate-respiring cells, suggesting that one of the isoforms of P450nor (P450norA) is in anaerobic mitochondrion of F. oxysporum and acts as nitric oxide reductase. Furthermore, these findings suggest the involvement of P450nor in nitrate respiration in mitochondria.

2.津下英明（徳島文理大）

Crystallization and Preliminary X-Ray Studies of the Ia Component of *Clostridium perfringens* Iota Toxin Complexed with NADPH

Journal of Structural Biology **126**, 175-177 (1999)

Hideaki Tsuge[1], Masahiro Nagahama[2], Tomohiro Nishimura[1], Yoshihiko Sakaguchi[2], Nobuhiko Katunuma[1], and Jun Sakurai[2]

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summary

A recombinant Ia component of *Clostridium perfringens* iota toxin, which ADP-ribosylates actin, was crystallized by the hanging drop vapor diffusion method using PEG4000 as a precipitating agent. The crystals were obtained in the presence of NADPH, which is similar to a real substrate, NADH, and belongs to the space group

P1 (a = 47.9 Å, b = 54.5 Å, c = 103.1 Å, α = 99.0°, β = 93.3°, and γ = 107.2°). The Matthews coefficient of native crystal was 2.7, assuming 2 mol/asymmetric unit. Native data were collected at 2.4-Å resolution. The results from a heavy-atom search showed that lanthanide ions (samarium, holmium) altered the molecular packing, judging from the unit-cell difference. The crystals also belonged to the space group P1 (a = 47.7 Å, b = 53.9 Å, c = 54.6 Å, α = 68.9°, β = 78.3°, and γ = 73.7°), which is consistent with only one molecule per asymmetric unit.

３．津下英明（徳島文理大）
Inhibition Mechanism of Cathepsin L-Specific Inhibitors Based on the Crystal Structure of Papain-CLIK148 Complex
Biochemical and Biophysical Research Communications **266**, 411-416 (1999)

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Summary
Papain was used as an experimental model structure to understand the inhibition mechanism of newly developed specific inhibitors of cathepsin L, the papain superfamily. Recently, we developed a series of cathepsin L-specific inhibitors which are called the CLIK series [(1999) FEBS Lett. 458, 6-10]. Here, we report the complex structure of papain with CLIK148, which is a representative inhibitor from the CLIK series. The inhibitor complex structure was solved at 1.7 Å resolution with conventional R 0.177. Unlike other epoxisuccinate inhibitors (E64, CA030, and CA074), CLIK148 uses both prime and nonprime sites, which are important for the specific inhibitory effect on cathepsin L. Also, the specificity for cathepsin L could be explained by the existence of Phe in the P2 site and hydrophobic interaction of N-terminal pyridine ring.

４．吾郷日出夫、宮野雅司（日本たばこ）
Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus
Structures **7**, 11, 1417-1426 (1999)

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Summary
Background: Hepatitis C virus (HCV) is the major etiological agent of hepatocellular carcinoma, and HCV RNA-dependent RNA polymerase (RdRp) is one of the main potential targets for anti-HCV agents. HCV RdRp performs run-off copying replication in an RNA-selective manner for the template-primer duplex and the substrate, but the structural basis of this reaction mechanism has still to be elucidated.
Results: The three-dimensional structure of HCV RdRp was determined by X-ray crystallography at 2.5 Å resolution. The compact HCV RdRp structure resembles a right hand, but has more complicated fingers and thumb domains than those of the other known polymerases, with a novel a-helix-rich subdomain (Afingers) as an addition to the fingers domain. The other fingers subdomain (β fingers) is folded in the same manner as the fingers domain of human immunodeficiency virus (HIV) reverse transcriptase (RT), another RNA-dependent polymerase. The ribose-recognition site of HCV RdRp is constructed of hydrophilic residues, unlike those of DNA polymerases. The C-terminal region of HCV RdRp occupies the putative RNA-duplex-binding cleft.
Conclusions: The structural basis of the RNA selectivity of HCV RdRp was elucidated from its crystal structure. The putative substrate-binding site with a shallow hydrophilic cavity should have ribonucleoside triphosphate (rNTP) as the preferred substrate. We propose that the unique αfingers might represent a common structural discriminator of the template-primer duplex that distinguishes between RNA and DNA during the replication of positive single-stranded RNA by viral RdRps. The C-terminal region might exert a regulatory function on the initiation and activity of HCV RdRp.

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Crystal Structure of Methionine Aminopeptidase from Hyperthermophile, *Pyrococcus furiosus*

J. Mol. Biol. **284**, 101-124 (1998)

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Summary
The structure of methionine aminopeptidase from hyperthermophile *Pyrococcus furiosus* (PfMAP) with an optimal growth temperature of 100°C was determined by the multiple isomorphous replacement method and refined in three different crystal forms, one monoclinic and two hexagonal, at resolutions of 2.8, 2.9, and 3.5 Å. The resolution of the monoclinic crystal form was extended to 1.75 Å by water-mediated transformation to a low-humidity form, and the obtained diffraction data used for high-resolution structure refinement. This is the first description of a eukaryotic

type methionine aminopeptidase structure.The PfMAP molecule is composed of two domains, a catalytic domain and an insertion domain, connected via two antiparallel β-strands. The catalytic domain, which possesses an internal 2-fold symmetry and contains two cobalt ions in the active site, resembles the structure of a prokaryotic type MAP from *Escherichia coli* (EcMAP), while the structure of the insertion domain containing three helices has a novel fold and accounts for a major difference between the eukaryotic and prokaryotic types of methionine aminopeptidase. Analysis of the PfMAP structure in comparison with EcMAP and other mesophile proteins reveals several factors which may contribute to the hyperthermostability of pfMAP: (1) a significantly high number of hydrogen bonds and ion-pairs between side-chains of oppositely charged residues involved in the stabilization of helices; (2) an increased number of hydrogen bonds between the positively charged side-chain and neutral oxygen; (3) a larger number of buried water molecules involved in crosslinking the backbone atoms of sequentially separate segments; (4) stabilization of two antiparallel β-strands connecting the two domains of the molecule by proline residues; (5) shortening of N and C-terminal tails and stabilization of the loop c,E by deletion of three residues.

6．三木邦夫（京大）
Crystal structure of a prokaryotic replication initiator protein bound to DNA at 2.6 Å resolution
The EMBO Journal **18,** 17, 4597-4607 (1999)
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Summary
The initiator protein (RepE) of F factor, a plasmid involved in sexual conjugation in *Escherichia coli*, has dual functions during the initiation of DNA replication which are determined by whether it exists as a dimer or as a monomer. A RepE monomer functions as a replication initiator, but a RepE dimer functions as an autogenous repressor. We have solved the crystal structure of the RepE monomer bound to an iteron DNA sequence of the replication origin of plasmid F. The RepE monomer consists of topologically similar N- and C-terminal domains related to each other by internal pseudo 2-fold symmetry, despite the lack of amino acid similarities between the domains. Both domains bind to the two major grooves of the iteron (19 bp) with different binding affinities. The C-terminal domain plays the leading role in this binding, while the N-terminal domain has an additional role in RepE dimerization. The structure also suggests that superhelical DNA induced at the origin of plasmid F by four RepEs and one HU dimer has an essential role in the initiation of DNA replication.

7．三木邦夫（京大）
Crystal Structure of Chitosanase from *Bacillus circulans* MH-K1 at 1.6-Å Resolution and Its Substrate Recognition Mechanism

The Journal of Biological Chemistry, **274**, 43, 30818-30825 (1999)

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Summary

Chitosanase from *Bacillus circulans* MH-K1 is a 29kDa extracellular protein composed of 259 amino acids. The crystal structure of chitosanase from B. *circulans* MH-K1 has been determined by multiwavelength anomalous diffraction method and refined to crystallographic R = 19.2% (R_{free} =23.5%) for the diffraction data at 1.6-Å resolution collected by synchrotron radiation. The enzyme has two globular upper and lower domains, which generate the active site cleft for the substrate binding. The overall molecular folding is similar to chitosanase from *Streptomyces sp.* N174, although there is only 20% identity at the amino acid sequence level between both chitosanases. However, there are three regions in which the topology is remarkably different. In addition, the disulfide bridge between Cys^{50} and Cys^{124} joins the β1 strand and the α7 helix, which is not conserved among other chitosanases. The orientation of two backbone helices, which connect the two domains, is also different and is responsible for the differences in size and shape of the active site cleft in these two chitosanases. This structural difference in the active site cleft is the reason why the enzymes specifically recognize different substrates and catalyze different types of chitosan degradation.

８．三木邦夫（京大）

Stimulation of Peroxidase Activity by Decamerization Related to Ionic Strength: AhpC Protein from Arn~hibacillus xylanus1

J. Biochem. 126, 313-319 (1999)

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Summary

AhpC protein, purified from Amphibacillus xylanus with a molecular mass of 20.8 kDa, protects cells against oxidation damage. The enzyme catalyses the reduction of hydroperoxides in cooperation with the 55 kDa flavoprotein,A. *xylanus* NADH oxidase (NADH oxidase-AhpC system). A. *xylanus* AhpC has two disulfide linkages between monomers and can act in the homodimer form. Gel-filtration column chromatography and dynamic light scattering (DLS) suggest that A. *xylanus* AhpC also forms a large oligomeric assembly (10-12 mers). A. *xylanus* AhpC was crystallized and X-ray diffraction data were collected to 3.0 Å. The self-rotation function revealed fivefold and twofold axes located perpendicularly to each other, suggesting that the molecular assembly of A. *xylanus* AhpC is composed of ten monomers. The oligomerization

of A. *xylanus* AhpC is affected by ionic strength in the DLS measurements. The H_2O_2 reductase activity of the A. *xylanus* NADH oxidase-AhpC system is also affected by ionic strength, and it was found that the decamerization of AhpC might be required for the activation of the NADH oxidase-AhpC system.

９．三木邦夫（京大）

Crystallization and preliminary X-ray diffraction studies of undecaprenyl diphosphate synthase *from Micrococcus luteus* B-P 26

Acta Cryst. **D55,** 1606-1607 (1999)

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Summary

Undecaprenyl diphosphate synthase from *Micrococcus luteus* B-P 26, one of the Z-prenyl chain-elongating enzymes, was crystallized using the sitting-drop vapour-diffusion method with ammonium sulfate and lithium sulfate as precipitants. The crystals belong to the monoclinic space group C2, with unit-cell parameters a = 127.2, b = 60.2. c = 75.7 Å. β = 105.6°. The crystals diffract X-rays to at least 2.2 Å resolution using synchrotron radiation and are suitable for high-resolution crystal structure analysis.

１０．三木邦夫（京大）

Ribulose Bisphosphate CarboxyIase/Oxygenase from the Hyperthermophilic Archaeon *pyrococcus kodakaraensis* KOD1 is Composed Solely of Large Subunits and Forms a Pentagonal Structure

J. Mol. Biol. **293**, 57-66, (1999)

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Summary

We previously reported the presence of a highly active, carboxylase-specific ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in a hyperthermophilic archaeon, *Pyrococcus kodakaraensis* KOD1. In this study, structural analysis of Pk-Rubisco has been performed. Phylogenetic analysis of Rubiscos indicated that archaeal Rubiscos, including Pk-Rubisco, were distinct from previously reported type I and type II enzymes in terms of primary structure. In order to investigate the existence of small subunits in native Pk-Rubisco, immunoprecipitation and native-PAGE

experiments were performed. No specific protein other than the expected large subunit of Pk-Rubisco was detected when the cell-free extracts of *P. kodakaraensis* KOD1 were immunoprecipitated with polyclonal antibodies against the recombinant enzyme. Furthermore, native and recombinant Pk-Rubiscos exhibited identical mobilities on native-PAGE. These results indicated that native Pk-Rubisco consisted solely of large subunits. Electron micrographs of purified recombinant Pk-Rubisco displayed pentagonal ring-like assemblies of the molecules. Crystals of Pk-Rubisco obtained from ammonium sulfate solutions diffracted X-rays beyond 2.8 Å resolution. The self-rotation function of the diffraction data showed the existence of 5-fold and 2-fold axes, which are located perpendicularly to each other. These results, along with the molecular mass of Pk-Rubisco estimated from gel filtration, strongly suggest that Pk-Rubisco is a decamer composed only of large subunits, with pentagonal ring-like structure. This is the first report of a decameric assembly of Rubisco, which is thought to belong to neither type I nor type II Rubiscos.

１１．三木邦夫（京大）

Purification, Crystallization, and Preliminary X-ray Crystallographic Analysis of Thermus thermophilus V1-ATPase B Subunit
Journal of Structural Biology 127, 79-82 (1999)
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Summary
The gene of V1-ATPase B subunit from the thermo- philic eubacterium Thermus thermophilus has been cloned and the protein overproduced in Escherichia coli. The purified protein, with a molecular weight of 53.2 kDa, was crystallized from 10% (w/v) polyethylene glycol 1000, 120 mM magnesium chloride, and 100 mM Na-tricine, pH 8.0, by the vapor diffusion method. The crystals diffracted X-rays beyond 3.5 Å on a synchrotron radiation source. The crystals belong to the monoclinic space group C2, with unit cell dimensions of a = 153.1 Å, b = 129.6 Å, c = 92.7 Å, and β = 100.3°. Assuming that three or four molecules are contained in an asymmetric unit, the VM value is calculated as 2.8 or 2.1 Å3/Da, respectively.

１２．甲斐泰（阪大）

Hyperthermostable Protein Structure Maintained by lntra and Inter-helix Ion-pairs in Archaeal O6- Methylguanine-DNA Methyltransferase
J. Mol. Biol. 292, 707-716 (1999)
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Summary
The crystal structure of O6-methylguanine-DNA methyltransferase (EC 2.1.1.63) of hyperthermophilic archaeon Pyrococcus kodakaraensis strain KODl (Pk-MGMT) was determined by single isomorphous replacement method with anomalous scattering (SIRAS) at 1.8 Å resolution. The archaeal protein is extremely thermostable and repairs alkylated DNA by suicidal alkyl transfer from guanine O6 to its own cysteine residue. Archaea constitute the third primary kingdom of living organisms, sharing characteristics with procaryotic and eucaryotic cells. They live in various extreme environments and are thought to include the most ancient organisms on the earth. Structural studies on hyperthermophilic archaeal proteins reveal the structural features essential for stability under the extreme environments in which these organisms live, and will provide the structural basis required for stabilizing various mesophilic proteins for industrial applications. Here, we report the crystal structure of Pk-MGMT and structural comparison of Pk-MGMT and methyltransferase homologue from Escherichia coli (AdaC, C-terminal fragment of Ada protein). Analyses of solvent-accessible surface area (SASA) reveals a large discrepancy between Pk-MGMT and AdaC with respect to the property of the ASA. In the Pk-MGMT structure, the intra-helix ion-pairs contribute to reinforce stability of α-helices. The inter-helix ion-pairs exist in the interior of Pk-MGMT and stabilize internal packing of tertiary structure. Furthermore, structural features of helix cappings, intra and inter-helix ion-pairs are found around the active-site structure in Pk-MGMT.

１３．甲斐泰（阪大）

Plausible phosphoenolpyruvate binding site revealed by 2.6Å structure of Mn2+-bound phosphoenolpyruvate carboxylase from Escherichiu coli
FEBS Letters 458, 93-96 (1999)

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Summary
We have determined the crystal structure of Mn2+-bound Escherichia coli phosphoenolpyruvate carboxylase (PEPC) using X-ray diffraction at 2.6 Å resolution, and specified the location of enzyme-bound Mn2+, which is essential for catalytic activity. The electron density map reveals that Mn2+ is bound to the side chain oxygens of Glu-506 and Asp-543, and located at the top of the α/β barrel in PEPC. The coordination sphere of Mn2+ observed in E. coli PEPC is similar to that of Mn2+ found in the pyruvate kinase structure. The model study of Mn2+-bound PEPC complexed with

phosphoenolpyruvate (PEP) reveals that the side chains of Arg-396, Arg-581 and Arg-713 could interact with PEP.

１４．甲斐泰（阪大）

Biochemisty 38, 13853- 13861 (1999)
Structure Comparison between Oxidized and Reduced Plastocyanin from a Fern, Dryopteris crassirhizoma
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Summary
The X-ray crystal structures of oxidized and reduced plastocyanin obtained from the fern Dryopteris crassirhizoma have been determined at 1.7 and 1.8 Å resolution, respectively. The fern plastocyanin is unique in the longer main chain composed of 102 amino acid residues and in the unusual pH dependence due to the π-π stacking interaction around the copper site [Kohzuma, T., et al. (1999) J. Biol. Chem. 274, 11817-11823]. Here we report the structural comparison between the fern plastocyanin and other plastocyanins from cyanobacteria, green algae, and other higher plants, together with the structural changes of fern plastocyanin upon reduction. Glu59 hydrogen bonds to the OH of Tyr83, which is thought to be a possible conduit for electrons, in the oxidized state. However, it moves away from Tyr83 upon reduction like poplar plastocyanin.

１５．山縣ゆり子（阪大）

Contribution of Hydrogen Bonds to the Conformational Stability of Human Lysozyme: Calorimetry and X-ray Analysis of Six Ser →Ala Mutants
Biochemistry 38, 6623-6629 (1999)
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Summary
To further examine the contribution of hydrogen bonds to the conformational stability of the human lysozyme, six Ser to Ala mutants were constructed. The thermodynamic parameters for denaturation of these six Ser mutant proteins were investigated by differential scanning calorimetry (DSC), and the crystal structures were determined by X-ray analysis. The denaturation Gibbs energy (AG) of the Ser mutant

proteins was changed from 2.0 to -5.7 kJ/mol, compared to that of the wild-type protein. With an analysis in which some factors that affected the stability due to mutation were considered, the contribution of hydrogen bonds to the stability (ΔΔGHB) was extracted on the basis of the structures of the mutant proteins. The results showed that hydrogen bonds between protein atoms and between a protein atom and a water bound with the protein molecule favorably contribute to the protein stability. The net contribution of one intramolecular hydrogen bond to protein stability (ΔGHB) was 8.9 ± 2.6 kJ/mol on average. However, the contribution to the protein stability of hydrogen bonds between a protein atom and a bound water molecule was smaller than that for a bond between protein atoms.

１６．山縣ゆり子（阪大）

A General Rule for the Relationship between Hydrophobic Effect and Conformational Stability of a Protein: Stability and Structure of a Series of Hydrophobic Mutans of Human Lysozyme

J. Mol. Biol. 280, 749-761 (1998)

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Summary

To get a general rule for the relationship between hydrophobic effect and conformational stability, five Ile to Val and nine Val to Ala mutants (3SS mutants) from 3SS (C77A/C95A) human lysozyme were constructed. As known from previous studies, the 3SS protein lacking a disulfide bond between Cys77 and Cys95 is destabilized by enthalpic factors, as revealed by a decrease of about 20 kJ/mol in the denaturation Gibbs energy change (ΔG) value, as compared to the wild-type protein, which has four disulfide bonds. In this study, the stabilities and structures of the 3SS mutants were determined by differential scanning calorimetry and X-ray crystal analysis, respectively, and compared with those of the mutants (4SS mutants) from the wild-type (4SS) protein published previously.

The stabilities of all the 3SS mutants, except for V110A-3SS were decreased as compared with that of the 3SS protein, coinciding with the results for the 4SS mutants. The change in the denaturation Gibbs energy change (ΔΔG) values of the 3SS mutants relative to the 3SS protein at the denaturation temperature (49.2°C) of the 3SS protein at pH 2.7 were similar to those of the equivalent 4SS mutants relative to the wild-type at 64.9°C.

The ΔΔG values of the 3SS mutants correlated with the changes in hydrophobic surface area exposed upon denaturation (ΔΔASAHP) for all of the hydrophobic residues when the effects of the secondary structure propensity were considered. This correlation is identical with that previously found for the 4SS mutants. The linear relation between ΔΔG and ΔΔASAHP for all of the hydrophobic residues with the same slope was found also for the mutants of T4 lysozyme already reported, indicating that this is a general relationship between changes in conformational stability and changes in ASA values of hydrophobic residues due to mutations.

１７．山縣ゆり子（阪大）

Experimental verification of the 'stability profile of mutant protein' (SPMP) data using mutant human lysozymes

Protein Engineering 12, 8, 663-672 (1999)

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Summary

The stability profile of mutant protein (SPMP) (Ota,M., Kanaya,S. and Nishikawa,K., 1995, J. Mol. Biol., 248, 733-738) estimates the changes in conformational stability due to single amino acid substitutions using a pseudo-energy potential developed for evaluating structure-sequence compatibility in the structure prediction method, the 3D-1D compatibility evaluation. Nine mutant human lysozymes expected to significantly increase in stability from SPMP were constructed, in order to experimentally verify the reliability of SPMP. The thermodynamic parameters for denaturation and crystal structures of these mutant proteins were determined. One mutant protein was stabilized as expected, compared with the wild-type protein. However, the others were not stabilized even though the structural changes were subtle, indicating that SPMP overestimates the increase in stability or underestimates negative effects due to substitution. The stability changes in the other mutant human lysozymes previously reported were also analyzed by SPMP. The correlation of the stability changes between the experiment and prediction depended on the types of substitution: there were some correlations for proline mutants and cavity-creating mutants, but no correlation for mutants related to side-chain hydrogen bonds. The present results may indicate some additional factors that should be considered in the calculation of SPMP, suggesting that SPMP can be refined further.

１８．山縣ゆり子（阪大）

Contribution of Intra- and Intermolecular Hydrogen Bonds to the Conformational Stability of Human Lysozyme

Biochemistry 38, 12698-12708 (1999)

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Summary

In globular proteins, there are intermolecular hydrogen bonds between protein and water molecules, and between water molecules, which are bound with the proteins, in addition to intramolecular hydrogen bonds. To estimate the contribution of these

hydrogen bonds to the conformational stability of a protein, the thermodynamic parameters for denaturation and the crystal structures of five Thr to Val and five Thr to Ala mutant human lysozymes were determined. The denaturation Gibbs energy (ΔG) of Thr to Val and Thr to Ala mutant proteins was changed from 4.0 to -5.6 kJ/mol and from 1.6 to -6.3 kJ/mol, respectively, compared with that of the wild-type protein. The contribution of hydrogen bonds to the stability (ΔΔGHB) of the Thr and other mutant human lysozymes previously reported was extracted from the observed stability changes (ΔΔG) with correction for changes in hydrophobicity and side chain conformational entropy between the wild-type and mutant structures. The estimation of the ΔΔGHB values of all mutant proteins after removal of hydrogen bonds, including protein-water hydrogen bonds, indicates a favorable contribution of the intra- and intermolecular hydrogen bonds to the protein stability. The net contribution of an intramolecular hydrogen bond (ΔGHB [pp]), an intermolecular one between protein and ordered water molecules (ΔGHB [pw]), and an intermolecular one between ordered water molecules (ΔGHB [ww]) could be estimated to be 8.5, 5.2, and 5.0 kJ/mol, respectively, for a 3 Å long hydrogen bond. This result shows the different contributions to protein stability of intra- and intermolecular hydrogen bonds. The entropic cost due to the introduction of a water molecule (ΔGH2O) could be also estimated to be about 8 kJ/mol.

１９．油谷克英（阪大）

The Unusually Slow Unfolding Rate Causes the High Stability of Pyrrolidone Carboxyl Peptidase from a Hyperthermophile, Pyrococcus furiosus: Equilibrium and Kinetic Studies of Guanidine Hydrochloride-Induced Unfolding and Refolding

Biochemistry 37, 17537-17544 (1998)

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Summary

To elucidate the energetic features of the anomalously high-level stabilization of a hyperthermophile pyrrolidone carboxyl peptidase (PfPCP) from a hyperthermophilic archaeon, Pyrococcus furiosus, equilibrium and kinetic studies of the guanidine hydrochloride (GuHCl)-induced unfolding and refolding were carried out with CD measurements at 220 nm in comparison with those from the mesophile homologue (BaPCP) from Bacillus amyloliquefaciens. The mutant protein of PfPCP substituted with Ser at both Cys142 and Cys188 (PfC142/188S) was used. The GuHCl unfolding for PfC142/188S and BaPCP was reversible. It was difficult to obtain the equilibrated unfolding curve of the hyperthermophile proteins at temperatures below 50 "C and pH 7, because of the remarkably slow rate of the unfolding. The unfolding for PfC142./188S attained equilibrium after 7 days at 60 "C, resulting in the coincidence between the unfolding and refolding curves. The Gibbs energy change of unfolding, ΔGH2O (56.6 kJ/mol), for PfC142/ 188S at 60 °Cand pH 7 was dramatically higher than that (7.6 kJ/mol) for BaPCP at 40 °C and pH 7. The unfolding and refolding kinetics for PfC142/188S and BaPCP at both 25 and 60 °C at pH 7 were approximated as a single exponential. The rate constant

in water (kuH20) of the unfolding reaction for PfC142/188S (1.6× 10-15 s-1) at 25°C and pH 7 was drastically reduced by 7 orders of magnitude compared to that (1.5× 10-8 s-l) for BaPCP, whereas the refolding rates (krH20) in water for PfC142/188S (9.3 ×10-2 s-1) and BaPCP (3.6×10-l s-l) at 25°C and pH 7 were similar. These results indicate that the greater stability of the hyperthermophile PCP was characterized by the drastically slow unfolding rate.

２０．油谷克英（阪大）

Evaluation of Some Factors that Contribute to Conformational Stability of a Protein Using Database of Stability/Structure

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Summary

The contribution of hydrophobic effects and hydrogen bonds to conformational stability of a protein was estimated using database of structure and stability changes due to mutations, which are obtained from a series of mutant human lysozymes. The magnitude of hydrophobicity of carbon atom (C), and the hydrophobicity of nitrogen and neutral oxygen atoms (N/O) could be estimated to be 0.178 kJ/mol/Å2, and -0.013 kJ/mol/Å2, respectively. The net contribution of an intramolecular hydrogen bond, an intermolecular one between protein and ordered water molecules, and an intermolecular one between ordered water molecules was estimated to be 8.5, 5.2, and 5.0 kJ/mol, respectively, for a hydrogen bond with 3 A length. The entropic cost due to the introduction of a water molecule could be also estimated to be about 8 kJ/mol.

２１．Zheng-jiong Lin (Institute of Biophysics. Academia Sinica, China)

Crystallization and preliminary X-ray analysis of human muscle creatine kinase

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Summary

Creatine kinase is a key enzyme in the energy homeostasis of cells and tissues with high and fluctuating energy demands. Human muscle MM creatine kinase is a dimeric protein with a molecular weight of ~43 kDa for each subunit. It has been crystallized by the hanging-drop vapor-diffusion method using 2-methyl-2.4pentanediol as precipitant. The crystals belong to the enantiomorphous space group P6222 or P6422 with cell parameters of a = b= 89.11 and c = 403.97Å. The asymmetric unit of the crystal contains two subunits. A data set at 3.3 Åresolution has been collected using synchrotron radiation.

22. Zheng-jiong Lin (Institute of Biophysics. Academia Sinica, China)
Structure of Active Site Carboxymethylated D-Glyceraldehyde-3-phosphate Dehydrogenase from Palinurus versicolor
J. Mol. Biol. 287, 719-725 (1999)
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Summary
The structure of active site carboxymethylated D-glyceraldehyde-3-phosphate dehydrogenase from Palinurus versicolor was determined in the presence of coenzyme NAD+ at 1.88 Å resolution with a final R-factor of 0.175. The structure refinement was carried out on the basis of the structure of holo-GAPDH at 2.0 Å resolution using the program XPLOR. The carboxymethyl group connected to Cys149 is stabilized by a hydrogen bond between its OZl and Cys149N, and charge interaction between the carboxyl group and the nicotinamide moiety. The modification of Cys149 induced conformational changes in the active site, in particular, the site of sulphate ion 501 (the proposed attacking inorganic phosphate ion in catalysis), and segment 208-218 nearby. Extensive hydrogen-bonding interactions occur in the active site, which contribute to the higher stability of the modified enzyme. The modification of the active site did not affect the conformation of GAPDH elsewhere, including the subunit interfaces. The structures of the green and red subunits in the asymmetric unit are nearly identical, suggesting that the half-site reactivity of this enzyme is from ligand-induced rather than pre-existing asymmetry. It is proposed that the carboxymethyl group takes the place of the acyl group of the reaction intermediate, and the catalytic mechanism of this enzyme is discussed in the light of a comparison of the structures of the native and the carboxymethylated GAPDH.

23. David I. Stuart (Oxford Univ.)
Structure and Dimerization of a Soluble Form of B7-1
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Summary
B7-l (CD80) and 87-2 (CD86) are glycoproteins expressed on antigen-presenting cells. The binding of these molecules to the T cell homodimers CD28 and CTLA-4 (CD152) generates costimulatory and inhibitory signals in T cells, respectively. The crystal structure of the extracellular region of B7-1 (sB7-l), solved to 3Å resolution, consists of a novel combination of two Ig-like domains, one characteristic of adhesion molecules and the other previously seen only in antigen receptors. In the crystal lattice, sB7-1 unexpectedly forms parallel, 2-fold rotationally symmetric homodimers. Analytical

ultracentrifugation reveals that sB7-1 also dimerizes in solution. The structural data suggest a mechanism whereby the avidity-enhanced binding of 87-l and CTLA-4 homodimers, along with the relatively high affinity of these interactions, favors the formation of very stable inhibitory signaling complexes.