

Thermus thermophilus HB8 のシステム生物学へ向けて: 結晶化・構造解析

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結晶化

① タンパク質の結晶化-Initial Screening-
96 x 5 = 480 conditions

- Hampton: Crystal Screen (48), Crystal Screen 2 (48), Index (96), SaltRX (96), Peg/Ion Screen (48), MembFac (48)
- Emerald BioStructures: Cryo I (48), Cryo II (48)

② タンパク質結晶化ロボット

TERA

HTS80
Miyatake, H. et al. (2005)
Acta Crystallogr. D61, 658-663

③ タンパク質結晶

Success rate of crystallization is very high.

データ収集、構造解析

④ SPring-8ビームライン

100μm程度 → Automatic crystal exchanger BL26B2

50μm以下 → Micro-sized Beam BL41XU

Collaborated with Dr. N. Shimizu (BL41XU)

⑤ BL26B2 -自動測定システム-

RIKEN Harima Structural Genomics Beamline BL26B2

Ueno, G. et al. (2004) J. Appl. Cryst. 37, 867-873

⑥ BL26B2 -メールインサービス-

遠隔操作測定

⑦ BL26B2の有効活用を目的とした実験室系回折ロボット

RAXIS VII diffractometer with the mounter robot

Automatic measurement 50 crystals / 10 h

Liquid N₂ auto-supplying machine

Developed by Oligomeric Protein Crystallography Team (Dr. Kunishima)

⑧ BL26B2測定用画面

センター画面, 測定条件決定画面, トレイ情報画面, 反射イメージ

⑨ BL26B2でのデータ収集 統計

| 実験期間 | 2006/05 ~ 2007/03 | |
|---------------------|-------------------|--|
| Inhouse Robot check | 7853 | |
| BL check | 1118 | |
| Dataset* | 455 | |
| Single | 274 | |
| MAD | 181 | |
| Dataset / Day** | 9 | |

* MAD dataset was counted as "one dataset".
** MAD dataset was counted as "three datasets".

⑩ BL41XUでの微小結晶測定実験

Crystal size : 25 x 25 x 40 μm
Data collection : 0 ~ 180°
Oscillation : 1°
Exposed time : 1 sec
Beam size : 25 x 25 μm
Shankar P. K. et al. (2007)
Acta Cryst. F63, 446-448

Max resolution = 1.9 Å

Single Data Collection
M.W.: 38830
No of residues: 352
Space Group: P2₁
Unit Cell parameters:
a=36.90 Å, b=132.96 Å, c=60.78 Å,
β=87.2°
Nmol in the asym: 1
Resolution: 1.9 Å
Phase determination: CNS v.1.1

⑪ 立体構造解析の例

立体構造解析に成功すると、機能未知タンパク質の約60%の機能発見が可能になった。

進捗状況

⑫ カテゴリー別進捗状況

Number of protein

Legend: others, Overexpression, Purification, Crystallization, Data collection, Structure

⑬ 進捗状況

| Category | Proteins |
|----------------------------|----------|
| (1) Plasmid construction | 2059 |
| (2) Protein overexpression | 1450 |
| (3) Purification | 944 |
| (4) Crystallization | 682 |
| (5) Data collection | 460 |
| (6) 3D structure | 360 |
| +106 (other groups) = 466 | |

Proteins

| Organism | Protein Structure Ratio (%) | | |
|--------------------------------------|-----------------------------|-----|----|
| <i>Thermus thermophilus</i> HB8 | 2213 | 466 | 21 |
| <i>Escherichia coli</i> K-12 | 4537 | 896 | 20 |
| <i>Thermotoga maritima</i> | 1876 | 263 | 14 |
| <i>Saccharomyces cerevisiae</i> | 5855 | 397 | 7 |
| <i>Bacillus subtilis</i> | 4106 | 257 | 6 |
| <i>Methanocaldococcus jannaschii</i> | 1803 | 67 | 4 |