CNR, Naples, Italy. [b]*Weizmann Institute, Rehovot, Israel.* [c]*Max Planck Institut, Hamburg, Germany.* E-mail: rberisio@chemistry.unina.it

Since the early days of ribosome research, the principal reaction of protein biosynthesis was localized in the large ribosomal subunit. Protein biosynthesis may be hampered by the occlusion of the exit tunnel, through which proteins emerge. This tunnel has a non-uniform diameter and contains grooves and cavities [1]. Crystal structures of complexes of the large ribosomal subunit from the eubacterium *Deinococcus radiodurans* with various polyketides (troleandomycin, telithromycin, rapamycin)[2,3] have shown that the exit tunnel is able to bind them with different fashions and that only some of those are capable to induce protein inactivation. We show that, among the three polyketides here analysed, rapamycin binds to a tunnel crevice that is located aside the typical macrolide-binding pocket and cannot occlude the exit tunnel. These structural results constitute the first example of a non-inactivating binding to the ribosome, thus suggesting that a necessary requirement for efficient antibiotic activity of macrolide-like compounds is their binding to the ribosome exit tunnel, in a manner that efficiently blocks the tunnel. Implications of polyketides binding to the ribosome large subunit will be discussed in the poster.

[1] Harms J., Schluenzen F., Zarivach R., Bashan A., Agmon I., Bartels H., Franceschi F., Yonath A., *Cell*, 2001, 679. [2] Berisio R., Harms J., Schluenzen F., Zarivach R., Hansen H., Fucini P., Yonath A., *J. Bacter.*, 2003, 4276. [3] Berisio R., Schluenzen F., Harms J., Bashan A., Auerbach T., Baram D., Yonath A., *Nat. Struct. Biol*., 2003, 366. [4] Amit M., Berisio R., Baram D., Harms J., Bashan A., Yonath A., *submitted*.

Keywords: ribosome, antibiotic, exit tunnel

P.04.09.7

Acta Cryst. (2005). A**61**, C228

Crystal Structure of a Conserved Hypothetical Protein TT1657 from *Thermus thermophilus* HB8

Mayumi Kanagawa[a], Akio Ebihara [a], Noriko Nakagawa[a,b], Gota Kawai[a,d], Shigeyuki Yokoyama[a,c,e], Seiki Kuramitsu[a,b,c], [a]*RIKEN Harima Institute at SPring-8.* [b]*Department of Biology, Graduate School of Science, Osaka University.* [c]*RIKEN Genomic Sciences Center.* [d]*Department of Life and Environmental Sciences, Chiba Institute of Technology.* [e]*Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan.* E-mail: kanagawa@spring8.or.jp

Thermus thermophilus HB8 is a good model to study the structure-function relationships. Because its proteins show high thermostablility, it is easier to solve their tertiary structures and the collection of structures will give sufficient information to study the structure-function relationship comparatively.

Here, we studied one of the *Thermus thermophilus* hypothetical proteins, TT1657, by the X-ray crystallography. The crystal structure was solved by the Se-MAD method. This protein forms dimer in the crystal and it is consistent with the result of the gel filtration experiment. Although BLAST search indicated that this protein has higher homology with some hypothetical proteins and weaker homology with phosphoesterases and phosphatases, DALI search shows high structural homology with some of phosphatases.

Keywords: phosphatases, thermostable proteins, crystal structures

P.04.09.8

Acta Cryst. (2005). A**61**, C228

Structural Characterization of Archaeal Elongation Factors

Adriana Zagari[a,d], A. Ruggiero[a], V. Granata[a], G. Raimo[b], M. Masullo[c], P. Arcari[a], L. Vitagliano[d], [a]*Università "Federico II", Napoli.* [b]*Università del Molise, Isernia.* [c]*Università "Magna Graecia", Catanzaro.* [d]*IBB-CNR, Napoli, Italy.* E-mail: zagari@unina.it

Elongation factors (EF) are enzymes that play a major role in protein biosynthesis. However, limited structural information is available on elongation factors isolated from archaea/eukarya [1,2]. We have undertaken structural studies on elongation factors isolated form the hyperthermophilic archaeon *Sulfolobus solfataricus*. The interest for these proteins is twofold. Indeed, they represent a valuable system to investigate structure/function relationships in archaeal/ eukaryal elongation factors and to study structure/stability correlations. Our previous investigations have provided insight into the function of *Ss*EF-1αand into the role played by the magnesium in the nucleotide exchange process. Our data also provided a preliminary mechanism for the exchange process in EF-1α̃ In order to better define this mechanism, we are currently performing structural investigations on the exchange factor *Ss*EF-1β̃Native and MAD data have been collected and the solution of the structure is in progress. Furthermore, the complex between *Ss*EF-1α and ¸ *Ss*EF-1β has been prepared for crystallographic investigations. Finally, a combined analysis by CD spectroscopy and molecular modeling has contributed to highlight the structural determinants of *Ss*EF-1αthermostability.

[1] Vitagliano L., Masullo M., Sica F., Zagari A., Bocchini V., *Embo. J.*, 2001, **20**, 5305. [2] Vitagliano L., Ruggiero A., Masullo M., Cantiello P., Arcari P., Zagari A., *Biochemistry*, 2004, **43**, 6630.

Keywords: elongation cycle, protein biosynthesis, thermostability

P.04.09.9

Acta Cryst. (2005). A**61**, C228

Engineering the Substrate Specificity and Catalysis from Crystal Structures of the Beta-subunit of Acyl-CoA Carboxylase

Ting-Wan Lin, Deborah Mitchell, Huy Pham, Shiou-Chuan Tsai, *Department of Molecular Biology and Biochemistry, University of California, Irvine, USA.* E-mail: tingwanl@uci.edu

The carboxylation of acyl-Coenzyme A is one of the key regulation checkpoints for the biosynthesis of fatty acids and polyketides. Acetyl-Co A carboxylases (ACC) and propionyl-CoA carboxylase (PCC) catalyze the carboxylation of acetyl- and propionyl-CoAs to generate malonyl- and methylmalonyl-CoA, respectively. Inhibitors of the ACCases have been identified as potential therapeutics for cancer and obesity, as well as herbicides and antibiotics. The crystal structures of the carboxyltransferase domain, AccB and PccB in *S. coelicolor*, are hexamers [1] that assemble into a ring shaped complex. The biotin-binding pocket has been identified where biotin and propionyl-CoA bind perpendicular to each other and are highly hydrophobic. Mutagenesis and kinetics studies of PccB and AccB allowed interconversion of their corresponding substrate specificity for acetyl-CoA, propionyl-CoA and butyl-CoA. The mutants structures show that dimer interaction is essential for enzyme catalysis, stability, and substrate specificity, which is highly conserved among biotin-dependent carboxyltransferases. ACCase mutants with relaxed substrate specificity can provide novel extender units, which can be fed into the polyketide biosynthesis pathway to generate "unnatural" natural products.

[1] Diacovich L., Mitchell D.L., Pham H., Gago G., Melgar M.M., Khosla C., Gramajo H., Tsai S.C., *Biochemistry*, 2004, **43(44)**, 14027-36.

Keywords: carboxylase, X-ray structure determination, fatty acid biosynthesis

P.04.10.1

Acta Cryst. (2005). A**61**, C228-C229

The Effects of Temperature and Radiation on Holo and Apo Ferritin Crystals

Robert J. Southworth-Davies[a], James W. Murray[a,c], Robin Owen[a], Enrique Rudiño-Piñera[b], Elspeth Garman[a], [a]*Department of Biochemistry, South Parks Road, Oxford, OX13QU, UK.* [b]*Instituto de Biotecnologia, Universidad Nacional Autonoma De Mexico, Cuernavaca, Morelos, C.P.62271, Mexico.* [c]*Current address: Institute for Cell and Molecular Biosciences,University of Newcastle, Newcastle-upon-Tyne, NE2 4HH,UK.* E-mail: elspeth@biop.ox.ac.uk

It is known that both temperature and radiation dose induce expansion of the unit cell of cryocooled macromolecular crystals [1]. Dose-induced increases are not thought to be caused by temperature changes in the crystal. We have investigated the nature of the dose and temperature induced unit cell expansion.

A series of datasets were collected at SRS Daresbury and ID14-4 at ESRF Grenoble on crystals of apo and holo ferritin at 100K. Further sets were collected on a second crystal of each type, but this time over