ESRF	**Experiment title:** Crystallographic Studies on Ribosomes	**Experiment number:** LS-1232
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Shifts: 30 per year	**Local contact(s):** B. Rasmussen E. Mitchel	*Received at ESRF:*

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Report:

I. The Small Ribosomal Subunits from Thermus termophilus and Their Complexes

The small subunit is of 0.85 mDa. It contains an RNA chain of about 1500 nucleotides (called 16S) and 20 different proteins. Treated and functionally activated crystals of these particles ($P4_12_12$ a=b=407 c=176 A) diffract currently to 3 Å. The systems studied by us are:

1. "Native" subunits. Since the best diffraction was obtained by the engineering of tungstenated- activated T30S crystals, the definition of "native" became flexible. Among the types that could serve as natives are WATIVES (a tungsten cluster treated natives) and Back-soaked Watives (BS).
2. Pre- and post-crystallization functionally activated subunits.
3. Pre-crystallization slightly crosslinked activated particles.
4. Heavy atom soaked watives and back-soaked crystals.
5. Heavy atom soaked untreated.
6. Pre-crystallization chemically modified particles, in which mono functional clusters (a tetra iridium or a tetra mercury) were covalently bound to the exposed cysteines.
7. Complexes of T30S with: (a) a mercurated analogue of the first 19 bases if the primer mRNA chain, containing the trigger sequence (Shine Dalgarno); (b) aminoglicoside antibiotics, such as edeine; (c) the translational initiation factor 3 (IF3); (d) cDNA oligomers, targeting exposed single stranded rRNA.

The higher resolution data were collected mainly at EFSR (Table I) yielded MIRAS maps. First at 4.5 Å (Tocilj et al., 1999) and later at 3.6 Å (manuscript in preparation). These maps show particles with a shape grossly similar to what seen by cryo electron microscopy. Over 900 bases of the 16S rRNA chain

were traced and 12 proteins were localized, among them two (S11 and S13) by direct labeling with heavy atom clusters. The known high resolution structures of eight proteins, namely S4, S5, S6, S7, S8, S15, S17 and S19 were placed in the map according to the available non crystallographic information, and tentative structures were extracted from the MIRAS map for proteins S13, S16 and S20.

In addition, the locations of three functional compounds were identified. These include: (a) the primer mRNA, containing the biosynthesis trigger (the Shine-Dalgarno sequence); (b) the binding of the translational initiation factor 3; (c) edeine, an antibiotic agent, targeting the decoding site. (d) docking experiments led to the identification of the locations of the A-, P- and E- tRNA binding sites; (e) the components that are likely to be involved in the intersubunit interface and in the decoding center, which is seen with remarkable clarity.

TABLE I: DATA COLLECTED AT ESRF (1998-99)

Period	Beamline	Crystals Collected W/Bs/C/M	HA
2.12-10.12.98	ID2	34	5
21.4-27.4.99	ID2	31	8
15.7-16.7.99	ID14-4	3	3
19.9.99	ID14-2	1	1
22.9-28.9.99	ID2	19	8

(W=wative, BS=back-soaked W, C=Complexes, M=modifide, HA=haevy atom soaks).

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Total number of exposed crystals=316, among them 113 used for data collection.

Because of the severe radiation decay, whenever possible, crystals were exposed in several positions in order to maximize the data obtained from individual crystals. A typical example is the complete data set of the complex of T30S with the mercurated mRNA analogue that was collected from 18 crystals, each exposed at 1-4 positions. The highest resolution measured from these crystal was 3.6 A. The typical resolution limits were 3.9-30A. Three crystals diffracted only to 4.5 A. A total rotation of 3-10 ° was collected from an individual crystal (about 1-3 ° per position). Rsym of the individual crystal-regions was 6-10% and between crystals = 9-12%.

II. The Large Ribosomal Subunits from Thermus termophilus and Haloarcula marismortui

The large subunit is of 1.45 mDa and contains RNA chains with a total of 3000 nucleotides and 34 different proteins. Further experiment on the crystals from the halophilic large subunits (H50S) led to phases at 5 Å resolution which yielded a rather fragmented map, similar to that published by the Yale group a few months ago. The most striking feature, a narrow contact area along the C axis, connecting the two halves of the unit cell ($C222_1$, a=211, b=300 and c=567 Å), may be the cause for the undesirable properties of this crystal form, mainly the low-isomorphism.

In order to increase the chances of obtaining high-resolution structure of the large subunit and, at the same time, to concentrate on the same source that yielded the crystals of the small subunit, we improved the crystals of T50S by antibiotics treatment. These crystals ($P4_12_12$ a=b=495 c=195 Å) diffract currently to 3.4 Å (compared to the previous 9 Å limit). A 4 Å native data set was collected at ID2.

The list of publication (past 18 months) is given on the other side of this document.

Ribosomal Crystallography, LS-1232: Publication list (past 18 months)

1. A. Yonath, J. Harms, H.A.S. Hansen, A. Bashan, F. Schluenzen, I. Levin, I. Koelln, A. Tocilj, I. Agmon, M. Peretz, H. Bartels, W.S. Bennett, S. Krumbholz, D. Janell, S. Weinstein, T. Auerbach, H. Avila, M. Piolleti, S. Morlang and F.Franceschi. Acta Cryst (1998) A54,945-955 Crystallographic Studies on the Ribosome, a large Macromolecular Assembly Exhibiting Severe Non-Isomorphism, Extreme Beam Sensitivity and No Internal Symmetry

2. S.Krumbholz, F.Schluenzen, J.Harms, H.Bartels, I.Koelln, K.Knaack, W.S.Bennett, P. Bhanumoorthy, H.A.S.Hansen, N.Volkmann, A.Bashan, I.Levin, A.Tocilj and A.Yonath, Periodicum Biologorum 100, 119-125 (1998) Ribosomal Crystallography, Cryo Protectants and Cooling Agents

3. J.Harms, A.Tocilj, I.Levin, I.Agmon, H.Stark, I.Koelln, M. van Heel, M. Cuff, F. Schluenzen, A. Bashan, F. Franceschi and A. Yonath. Structure, (1999) 7, 931-941 Elucidating the medium resolution structure of the ribosome: an interplay between electron-cryo microscopy and X-ray crystallography

4. S.Weinstein, W.Jahn, C.Glotz, F.Schluenzen, I.Levin, D.Janell, J.Harms, I.Koelln, H.A.S.Hansen, M. Gluehmann, W.S.Bennett, H.Bartels, A.Bashan, I.Agmon, M.Kessler, M.Pioletti, H.Avila, K.Anagnostopoulos, M. Peretz, T. Auerbach, F. Franceschi and A.Yonath. J. Struct. Biol. 127, 141-151 (1999). Metal compounds as tools for the construction and the interpretation of medium resolution maps of ribosomal particles

5. F.Schluenzen, I.Koelln, D.Janell, M.Gluehmann, I.Levin, A.Bashan, J.Harms, H.Bartels, T.Auerbach, M.Pioletti, H.Avila, K.Anagnostopoulos, H.A.S.Hansen, W.S.Bennett, I.Agmon, M.Kessler, A.Tocilj, M.Peretz, S.Weinstein, F.Franceschi and A. Yonath. J. Syn. Radiation. 6, 928-941 (1999) The identification of selected components in electron density maps of prokaryotic ribosomes at 7 A resolution

6. A.Tocilj, F. Schluenzen, H.A.S. Hansen, A. Bashan, D. Janell, M. Gluehmann, H. Bartels, J. Harms, A. Agmon, F. Franceschi, and A. Yonath. PNAS, 96, 14252-14257 (1999). The small ribosomal subunit from Thermus thermophilus at 4.5 A resolution: pattern fitting and the identification of functional sites

7. T.Auerbach, M. Pioletti, H. Avila, K. Anagnostopoulos, S. Weinstein, F. Franceschi & A.Yonath Biomolecular Structure and Dynamics, (1999) In the press. Genetic and biochemical manipulation of the small ribosomal subunit from T. thermophilus HB8

8. H. Bartels, M. Gluehmann, D. Janell, F. Schluenzen, A. Tocilj, A. Bashan, I. Levin, H.A.S. Hansen, J. Harms, M. Kessler, M. Pioletti, T. Auerbach I. Agmon, H. Avila, M. Simitsopoulou, S. Weinstein, M. Peretz, W.S. Bennett, F. Franceschi and A. Yonath. Cellular and Molecular Biology (2000) in the press. Targeting exposed RNA regions in crystals of the small ribosomal subunits at medium resolution

9. D.Janell, A.Tocilj, I.Koelln, F.Schluenzen, M.Gluehmann, H.A.S.Hansen, J.Harms, A.Bashan, I. Agmon, H.Bartels, M.Kessler, S.Weinstein, F.Franceschi & A.Yonath (2000) In Polyoxometalate Chem. with Interdisciplinary Aspects: From Topology to Industrial Applications (M. Pope and A. Mueller, Eds.) Kluwer Academic Publishers, in the press. Ribosomal crystallography and heteroplytungstate

10. A.Bashan, M.Pioletti, H.Bartels, D.Janell, F.Schluenzen, M.Gluehmann, I.Levin, J. Harms, H.A.S. Hansen, A.Tocilj, T.Auerbach, H.Avila, K.Anagnostopoulos, M.Simitsopoulou, M. Peretz, W.S. Bennett, I.Agmon, M.Kessler, S.Weinstein, F.Franceschi and A.Yonath (2000) ASM publications, in the press. The identification of selected ribosomal components in crystallographic maps of prokaryotic ribosomal subunits at medium resolution