LS1792 - Ribosomal crystallography

EXPERIMENTAL REPORT

1. Highlights - 2001-2

During the last two years we determined the high-resolution structures of both ribosomal subunits: the small one from *Thermus thermophilus* (T30S) (Schluenzen et al., 2000) and the large from *Deinococcus radiodurans* (D50S) (Harms et al., 2001). The latter is a mesophilic robust bacterium that shares over 80% identity with *E. coli*, a new addition to the sources suitable for ribosomal crystallography, which was found to be extremely useful. We also determined the structures of several complexes of both subunits with clinically relevant antibiotics, ligands, inhibitors and substrate analogs (Pioletti et al., 2001, Schluenzen et al., 2001, Zarivach et al., 2001, Auerbach et al., 2002, Schluenzen et al., 2002), and revealed an internal two-fold symmetry within the active site. These led to a proposed mechanism of peptide-bond formation, including proton transfer mediated by water or hydrated magnesium (manuscript in preparation). This mechanism is consistent with earlier suggestion that the ribosome provides the frame for accurate orientation of the tRNA molecules rather than participating in the chemical enzymatic activity, as proposed by the Yale group (Nissen et al., 2000), and was widely challenged**.**

Analysis of the interactions of a dozen ribosomal antibiotics studied by us illuminated various modes of their action. Thus antibiotics inhibit protein biosynthesis by reducing the decoding accuracy, limiting conformational mobility, interference with substrate binding and hindrance of the progression of growing proteins (Pioletti et al., 2001, Schluenzen et al., 2001, Auerbach et al., 2002). Our results shed light also on antibiotic selectivity and resistance. The uniqueness of our studies stems from the usage the ribosomes of an eubacterium resembling pathogens rather than ribosomes form archaea, used by the Yale group, which shares properties of eubacteria and eukaryotes. Hence our results rationalized a large volume of empiric observations, provided insight into resistance and paved the way for structural based drug design. Consequently, several pharmaceutical companies approached us and initiated collaborations or licensed our coordinates. Among these is Rib-X, the company founded recently by the Yale group.

The structures of both the large and the small ribosomal subunits determined by us contain all features involved in ribosomal activities, contrary to the 2.4 A structure of the halophilic large subunit determined by the Yale group, in which these structural elements are disordered. Comparisons of the structures determined by us with the lower resolution structure of the entire ribosome (Santa Cruz structure) identified flexible features and suggested possible ways for their function. These include how intersubunit bridges may be formed, how the tRNA molecules may exit the ribosome and how translocation can take place, major functional aspects of protein biosynthesis not seen before (Harms et al., 2001, Gluehmann et al, 2001, Yonath 2002).

The site of peptide bond formation is adjacent to the entrance of a tunnel along which the nascent proteins progress until they emerge out of the ribosome. This tunnel was assumed to provide a passive path for exporting smoothly all protein sequences. However, recently it was suggested to be involved in elongation arrest. Consistent with these findings, our studies revealed the dynamic capabilities of the nascent protein exit tunnel and correlated it with elongation arrest and sequence discrimination (manuscript in preparation), thus providing the only structural insight for the participation of the ribosome in sub-cellular regulation.

Data collection summary

**General**

In all experiments we used a fixed wavelength, of about 0.9-1 A. All experiments were performed at cryo temperature form shock frozen crystals in liquid propane. The rotation range was 0.1°, with exposure time of approximately 10-20 second per rotation. Incomplete data from a specific beamtime could usually be merged with data collected in another beamtime.

**Crystal systems**

T30S, $P4_12_12$; a=b=407 c=176 A
D50S I222; a=179, b=409, c=696 A
H50S C222; a=204, b=300, c=570 A
T50S, $P4_12_12$; a=b=496 c=194 A

**Crystal quality**

35% of the tested T30S crystals, including native (called "wative" since they include tungsten clusters as stabilizers) or complexes with factors or antibiotics, and 65% of the D50S crystals were used for data collection.

**Crystal decay**

T30S are very sensitive to irradiation. Typically we could obtain up to 1-2 degrees from a single location in the crystal.
Using a focused beam with a cross-section smaller than the crystal, and being able to translate the crystal once it decayed, we sometimes could collect 6-10 degrees from a single crystal.
TD50S crystals are much more stable in the beam. Typically we could collect 25-40 degrees from a single crystal. In a few cases we reached what could be lead to a complete set (namely 80-90 degrees). These are mentioned below.

## BEAM-TIMES REPORTS

**ID14-2: Data sets collected 14.-20.6.00**

Data were collected from 19 crystals of a complex of T30S with a heavy atom (called HA3), diffracting to 3.1-3.4 A. The highest amount of data collected from a single crystal was 13 deg, and the lowest 2 degrees. The R merge was 8-12% and the overall completeness higher than 90%.

**ID14-2: Data sets collected 7-12.2.01**

Data were collected from 4 crystals of three complexes of T30S, each with another factor or its segment, diffracting to app 4 A. The highest amount of data collected from a single crystal was 25 degrees, and the lowest 11.2 degrees. The R merge was 9-13%. Completeness = 96%.
About half a data set was collected from a crystal H50S to 3.2 A resolution. R merge 18.7%.

**ID14-4: Data sets collected 12.-16.2.01**

Data were collected from 5 crystals of complexes of T30S with a mixture of two factors, diffracting to app 3.4 A. The highest amount of data collected from a single crystal was 18 degrees, and the lowest 9 degrees. The R merge was 10-14%. Overall completeness = 81%.
Data were collected from 3 crystals of native D50S, diffracting to app 3.4 A and from a crystal of its complex with antibiotic #11. The highest amount of data collected from a single crystal was 98 degrees, and the lowest 56 degrees. The R merge was 10-14%. Completeness for each ~65%.

**ID14-2: Data sets collected 25.-27.4.01**

Data were collected from 3 crystals of native D50S, diffracting to app 3.3 A and from a crystal of its complex with a heavy atom derivative (HA4). The highest amount of data collected from a single crystal was 88 degrees, and the lowest 14 degrees. The R merge was 8-14%. Completeness for native >75% and for HA4 72%.

**ID14-4: Data sets collected 27.-30.4.01**

Data were collected from 9 crystals of complexes of D50S with a four different antibiotics (#10, #11, #13 and #1), diffracting to 3.1-3.4 A. The highest amount of data collected from a single crystal was 184 degrees, and the lowest 24 degrees. The R merge was 8-17%. Completeness of #14 was 94%, #13=65%, #10 = 90% and #11 = 43%.

**ID14-2: Data sets collected 17.-18.9.01**

Data were collected from 4 crystals of T50S, two crystals of D50S in complex with an antibiotics (#9) and four crystals of D50S with a substrate analog (SA1), diffracting to 3.1-3.4 A.
Data collected from T50S were found to be unusable above 4 A.
The highest amount of data collected from a single crystal of the antibiotic complex with D50S was 36 degrees. The R merge was 10-12%. Completeness = 69%.
The highest amount of data collected from a single crystal of the substrate analog complex with D50S was 34 degrees. The R merge was 9-13%. Completeness = 79%.

**ID14-4: Data sets collected 19.-22.9.01**

Data were collected from 14 crystals of complexes of D50S with a five different antibiotics (#31, #6, #30 #28 and #31g), diffracting to 2.9-3.4 A. The highest amount of data collected from a single crystal was 80 degrees, and the lowest 6 degrees. The R merge was 8-13%. Completeness of #31 was 55%, #6 = 36%, #30 = 44%, #28 = 24% and #31g = 27%.

**ID14-4: Data sets collected 9.-11.2.02**

Data were collected from 9 crystals of complexes of D50S with a six different antibiotics (#31, #43, #45 #39 #28 and #7/8), diffracting to 3.1-3.4 A. The highest amount of data collected from a single crystal was 69 degrees, and the lowest 11 degrees. The R merge was 9-14%. Completeness of #31 was 67%, #43 = 38%, #45 = 89%, #39 = 54% and #7/8 = 47%.

## ID14-2: Data sets collected 11.-12.2.02

Data were collected from 6 crystals of complexes of D50S with a four different antibiotics (#10, #46, #45 and #7/8), diffracting to 3.3-3.6 A. The highest amount of data collected from a single crystal was 92 degrees, and the lowest 13 degrees. The R merge was 9-14%. Completeness of #10 was 32%, #46 = 78%, #45 = 91%, and 7/8 = 75%.
35% of a data set were collected from 1 crystal of a complex of T30S with a small subunit in-activator, diffracting to 4.3 A. These data were found to be not useful even at low resolution.

## List of publications


**1.** T.Auerbach, A.Bashan, J. Harms, F. Schluenzen, R. Zarivach, H. Bartels, I. Agmon, M. Kessler, M. Pioletti, F. Franceschi and A. Yonath, **Antibiotics Targeting Ribosomes: Crystallographic Studies,** *Curr Drug Targets - Infectious Disorder*s, 2, 169-86 (2002)

**2.** A. Yonath, **The search and its outcome: high-resolution structures of ribosomal particles from mesophilic, thermophilic and halophilic bacteria at various functional states,** *Annu Rev Biophys Biomol Struct,* 31, 257-73 (2002)

**3.** R. Zarivach, A. Bashan, F. Schluenzen, J. Harms, M. Pioletti, F. Franceschi and A. Yonath, **Initiation and inhibition of protein biosynthesis – studies at high resolution**, *Current Protein and Peptid Science*, 3, 55-65 (2002)

**4.** A. Yonath**, High-resolution structures of large ribosomal subunits from mesophilic eubacteria and halophilic archaea at various functional states,** *Current Protein & Peptide Science* 3, 67-78 (2002)

**5.** A. Bashan, I. Agmon, R .Zarivach, F. Schluenzen, J. Harms, M. Pioletti, H. Bartels, M. Gluehmann, H.A. Hansen, T. Auerbach, F. Franceschi & A. Yonath, **High resolution structures of ribosomal subunits: initiation, inhibition and conformational variability,***Cold Spring Harb Symp Quant Biol, 66,* 43-56 (2001)

**6.** M.Gluehmann, R.Zarivach, A.Bashan, J.Harms, F.Schluenzen, H.Bartels, I.Agmon, G.Rosenblum, M.Pioletti, T.Auerbach, H.Avila, H.A.Hansen, F.Franceschi & A.Yonath, **Ribosomal crystallography: from poorly diffracting micro-crystals to high resolution structures,** *Methods*, 25, 292–302 (2001)

**7.** F. Schluenzen, R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath and F. Franceschi, **Structural basis for the interaction of chloramphenicol, clindamycin, and macrolides with the peptidyl transferase center in eubacteria**, *Nature*, 413, 814-21 (2001)

**8.** J. Harms, F. Schluenzen, R. Zarivach, A. Bashan, S. Gat, I. Agmon, H. Bartels, F. Franceschi and A. Yonath**, High resolution structure of the large ribosomal subunit from a mesophilic eubacterium**, *Cell,* 107, 679-88 (2001)

**9.** M.Pioletti, F.Schluenzen, J.Harms, R.Zarivach, M.Gluehmann, H. Avila, A. Bashan, H. Bartels, T.Auerbach, C.Jacobi, Hartsch T, A. Yonath and F. Franceschi, **Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3**, *EMBO J*, 20, 1829-39 (2001)

**10.** F. Schluenzen, A. Tocilj, R. Zarivach, J. Harms, M. Gluehmann, D. Janell, A. Bashan, H. Bartels, I. Agmon, F. Franceschi and A. Yonath, **Structure of functionally activated small ribosomal subunit at 3.3 A resolution**, *Cell*, 102, 615-23 (2000)

**11.** H. Bartels, M. Gluehmann, D. Janell, F. Schluenzen, A. Tocilj, A. Bashan, I. Levin, H.A. Hansen, M. Kessler, M. Pioletti, T. Auerbach, I. Agmon, W.S. Bennett, F. Franceschi and A. Yonath, **Targeting exposed RNA regions in crystals of the small ribosomal subunits at medium resolution,** *Cell Mol Biol (Noisy-le-grand),* 46, 871-82 (2000)

# The Large Ribosomal Subunit from *Deinococcus Radiodurans*