



	Experiment title: Complex of spliceosomal [U4/U6.U5]-tri-snRNP specific 15.5K protein and U4 snRNA oligoribonucleotide	Experiment number: LS-1297
Beamline:	Date of experiment: from: 25-Feb-99 to: 27-Feb-99	Date of report: 03-Aug-99
Shifts: 6	Local contact(s): Vivian Stojanoff	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

***Dr. Ralf Ficner**

***Dr. Klaus Reuter**

***Ivan Vidovic**

IMT, University of Marburg

Emil-Mannkopff-Strasse 2

D-35037 Marburg

Germany

Report:

The spliceosome undergoes numerous structural changes during pre-mRNA splicing especially concerning the U4 and U6 snRNAs. snRNP-proteins interacting directly with these snRNAs are of particular interest, because of their potential role in these structural rearrangements.

Recently, the human [U4/U6.U5] tri-snRNP specific protein U4-15.5kD, which specifically binds to the 5'-stem-loop of U4 snRNA, was identified.

We have crystalized the protein-RNA complex and performed a MAD experiment with a selenomethionylated U4-15.5kD protein using four different wavelengths. We have also collected a dataset of a native crystal.

Dataset	Wavelength	Completeness	linear R-fac	Resolution
MAD λ_1	0.9790	88.2 %	0.064	2.9 Å
MAD λ_2	0.9791	88.1 %	0.056	2.9 Å
MAD λ_3	0.9184	84.7 %	0.058	2.9 Å
MAD λ_4	0.9919	88.3 %	0.055	2.9 Å
native	0.9919	86.6 %	0.064	2.6 Å

The space group of the selenomethionyl U4-15.5kD crystal was $P2_12_12_1$ with cell constants $a=45.76$ Å $b=55.29$ Å $c=146.73$ Å. The unit cell dimensions of the non-isomorphous native crystal were $a=45.43$ $b=56.34$ $c=163.38$.

The protein-RNA complex has a total molecular weight of 22kDa. The crystal contains two complexes in the asymmetric unit. Each complex contains only one selenomethionine resulting in two selenium atoms per asymmetric unit. The selenium sites were identified using *Solve* and phases were calculated using *Sharp*. The protein exhibits a novel α/β -fold and binds to the strongly bent internal loop of the U4-snRNA 5'-stem loop. The structure is currently in the process of refinement.