ESRF	**Experiment title:** Structural studies of the human mitochondrial deoxyribonucleotidase	**Experiment number**: LS-2170
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Shifts: 3	**Local contact(s)**: Dr. Ferrer	*Received at ESRF:*

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Report: Structural studies of the human mitochondrial deoxyribonucleotidase

One dataset of deoxyribonucleotidase with thymidine and berylliumtrifluoride was collected that was included in an article published october 2002. See below for abstract and datacollection statistics.

-dNT-2 with thymidine and Berylliumtrifluoride:

Rinaldo-Matthis, A., C. Rampazzo, et al. (2002). "Crystal structure of a human mitochondrial deoxyribonucleotidase." Nat Struct Biol 9(10): 779-87.

5'-nucleotidases are ubiquitous enzymes that dephosphorylate nucleoside monophosphates and participate in the regulation of nucleotide pools. The mitochondrial 5'-(3') deoxyribonucleotidase (dNT-2) specifically dephosphorylates dUMP and dTMP, protecting mitochondrial DNA replication from excess dTTP.

We have solved the structure of dNT-2, the first determined structure of a mammalian 5'-nucleotidase. The structure reveals a relationship to the HAD family, with a phosphoserine phosphatase as the closest neighbor. A structure-based sequence alignment of dNT-2 with other 5'-nucleotidases also suggests a common origin for these enzymes.

The structure of dNT-2 has been studied in complex with bound phosphate and beryllium trifluoride plus thymidine as model for a phosphoenzyme product complex. Based on these structures, determinants for substrate specificity recognition and the catalytic action of dNT-2 are outlined.

Rinaldo-Matthis, A., C. Rampazzo, et al. (2002). "Crystal structure of a human mitochondrial deoxyribonucleotidase." Nat Struct Biol 9(10): 779-87.

TABLE 1 **Data statistics**

Data set	Thym
Wavelength (Å)	1.106
Space group	$P4_32_12$
a=,b=	74 Å
c=	106 Å
Resolution (Å)	20-2.8
I/σ(I)	8.5
Completeness(%)[1]	(99.8)
overall[1]	99.9
R_{sym}(%)[1,2]	12.2(34)
Avg. Redundancy	9.3
Resolution range	20-2.8
Reflections (working/test)	6616/791
Rcryst/Rfree[3]	14.2/20.6
Mean B-factors (Å^2)	45.8
Residues	193

Report: Time-resolved studies of oxygen activation of the ribonucleotide reductase R2 subunit.

One dataset collected on a hydrogenperoxide soaked crystal of the ribonucleotide reductase R2 subunit from *Chlamydia trachomatis*.

20-2,7 Å resolution
R-merge 0,072
Spacegroup $P4_32_12$
Cell parameters 86,5 86,5 185,8 90 90 90

The role of protein R2 is the generation of a stable tyrosyl radical via the reductive cleavage of dioxygen at a di-iron carboxylate site. The tyrosine residue carrying the radical in R2 is conserved among all sequenced R2 proteins with the exception of the *Chlamydia* family, which instsead of a tyrosine has a phenylalanine at this position (*Chlamydia trachomatis* F127, *E.coli* Y122).

Report: Class II Ribonucleotide reductase phasing project

Ribonucleotide reductases (RNRs) are essential for all life by virtue of catalysing the only dedicated step of deoxyribonucleotide synthesis, the reduction of ribonucleotides to deoxyribonucleotides. They all use radical chemistry and all are allosterically regulated [1]. RNRs can be divided into three classes based on their mechanism of radical generation [2]. Class I RNRs are oxygen-dependent and generate a stable tyrosyl radical by activation of molecular oxygen at the di-iron centre of an activating protein. Class III RNRs are strictly anaerobic and generate a stable glycyl radical by homolytic cleavage of S-adenosylmethionine at a [4Fe-4S] centre, again in a separate activating protein unrelated to that of class I. **Class II RNRs** generate a transient

5’-deoxyadenosyl radical by homolytic cleavage of the Co-C bond in adenosylcobalamin (adocob), which is bound directly to the reductase.

As a part of the phasing project of the class II ribonucleotide reductase from *Thermotoga maritima*, a 100 % complete SAD dataset to around 2.9Å was collected at the LIII edge of Iridum. However, the dataset did not contribute to the phasing effort as the occupancy of the $IrCl_2$ appeared to be very low.