ESRF	**Experiment title:** Time-resolved crystallography of the oxygen activation/radical generation reaction in ribonucleotide reductase R2	**Experiment number**: LS-1854
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Shifts: 3	**Local contact(s)**: Dr. Jamin	*Received at ESRF:*

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Report: Time-resolved crystallography of the oxygen activation of R2

The mechanism of oxygen activation at diiron sites is believed to be shared between the diiron-carboxylate proteins such as ribonucleotide reductase R2, methane monooxygenase and stearoyl-acyl carrier protein desaturase. For this reason it is of profound importance for medical as well as industrial applications.
Our aim with the project is to understand the structural basis for this complicated mechanism which includes several intermediates that have been isolated and studied by various spectroscopic methods.

We seek to solve structures of the intermediates in the oxygen activation and radical generation mechanism of ribonucleotide reductase R2. Data has been collected on wt as well as mutant *E. coli* R2 crystals in which the reaction has been quenched at different timepoints. We obtained some 15 complete datasets the resolution was generally 1.8-2.4Å and the R-merges 0.06-0.10.
We have also collected data on a novel R2 from *Chlamydia trachomatis* with suitable properties for these studies. Data has been collected on wt protein crystals that has undergone various redox experiments. 3 complete datasets, resolution 2.7; 1.9 and 3.0Å, R-merges 0.07; 0.07 and 0.12

Report: Structural studies of human mitochondrial dNT2

One dataset of the deoxyribonucleotidase (dNT-2) enzyme with an inhibitor was collected. The structure of dNT-2 with the inhibitor PMcP-U was included in an article submitted to Molecular Pharmacology. For abstract and details of data collection see below.

-dNT with PMcP-U

Crystal structure of the mitochondrial deoxyribonucleotidase in complex with two specific inhibitors
Agnes Rinaldo-Matthis[1)], Chiara Rampazzo [2)], Jan Balzarini[3)] Peter Reichard[2,4)], Vera Bianchi[2)] and Pär Nordlund[1)*] Submited to Molecular Pharmacology

Mono-phosphate nucleotidases are enzymes that dephosphorylate nucleotides to their corresponding nucleoside. They play potentially important roles in controlling the activation of nucleotide-based drugs and inhibitors in viral infections or cancer cells. The human mitochondrial deoxyribonucleotidase (dNT-2) dephosphorylates deoxy thymidine and uridine monophosphates. We here describe the high resolution structures of the dNT-2 enzyme in complex with two potent phosphonate inhibitors, DPB-T((S)-1-[2-Deoxy-3,5-O-(1-phosphono)benzylidene-β-D-*threo*-pentofuranosyl]thymine) at 1.6 Å resolution and PMcP-U (±-1-trans-(2-phosphonomethoxycyclopentyl)uracil) at 1.4 Å resolution respectively. The linear mixed competitive inhibitor DPB-T and the competitive inhibitor PMcP-U both bind in the active site of dNT-2 but in distinctly different binding modes. The pyrimidine part of the inhibitors binds with very similar hydrogen bond interactions to the protein but with their phosphonate groups in different binding sites when compared to each other, as well as to the previously determined position for phosphate binding to dNT-2. Together these phosphate/phosphonate binding sites describes what might constitute a functionally relevant phosphate entrance tunnel to the active site. The structures of the inhibitors in complex with dNT-2, being the first such complexes of any nucleotidase, might provide important information for the design of more specific inhibitors which could be useful for controlling the activation of nucleotide based drugs or to study the *in vivo* function of nucleotidases using chemical knockout.

Crystallographic data collection and refinement statistics

	PMcP-U
Spacegroup $P4_32_12$	
Unit cell dimensions (Å)	
a	73.6
b	73.6
c	105.7
Resolution	30-1.4
No. of observations	209410
No of unique reflections	57136
R_{merge}(last shell)	5.7(31)
Completeness(%)	98
Working R-value	13.3
No of reflections working set	40132
Free R-value	16.5
No of reflections test set	4518
Rms deviation from ideal geometry of final models	
Bond lengths (Å)	0.02
Bond angles (deg)	1.8
No residues	195

Report: Initial trials of heavy atom derivatives on *Thermotoga maritima class II* Ribonucleotide reductase

Datasets were collected on crystals soaked with different mercury and platnium compounds.
MeHg: Resolution 20-3.1, 78.1% complete, redundancy 1.8, Rmerge 6.3(32.1)
Pt: Resolution 20-2.92 , 96% complete, redundancy 2.4

We also screened extensively for functional heavy-atom derivatives.