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. Leonard	*Received at ESRF:*

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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. There is thus no accessory activating protein. In all three classes of RNR, the stable (class I and III) or transient (class II) radical is transferred to a cysteine residue in the active site, which generates a cysteinyl radical essential for abstraction of a hydrogen atom from the substrate in the first step of the reaction mechanism. The class II is the only RNR class where no stable radical is stored; instead a transient radical is generated, most likely in close proximity to the active site. The C-Co bond is reportedly regenerated and adenosylcobalamin dissociates from the enzyme after each turnover.

The crystals we have obtained of class II ribonucleotide reductase from Thermotoga maritima are normally of spacegroup $P2_1$ with a pseudo C2 symmetry. Upon soaking with heavy atoms the rule has been that they shift to a pure C2 symmetry. This has been problematic in the evaluation of our derivatives. The following datasets were collected during this experiment:

Au LIII edge

Peak dataset 180 degrees with a resolution: 20-3.0, 96.7% complete, redundancy 3.7 Rmerge: 7.2(20.1)

Ir LIII edge

Data collection time for each dataset was about 3-4 hours since we had to attenuate the beam significantly to prevent radiation damage (this from previous experience of the behaviour of the crystals at ID14-2 and ID14-4).
180 degrees/dataset with an osc. angle of 0.7 was collected on peak, inflectionpoint and remote. Resolution 30-2.7. 99.7 % complete, multiplicity 4.4
Peak dataset Rsym 6.2(30.2)
Inflection point dataset Rsym 7.2(35.5)
Remote dataset Rsym 7.6 (25.6)
This irdium data unfortunately gave only very weak phase information and only the molecular envelope could be discerned in spite of giving convincing initail experimental maps using SOLVE.

An additional Peak dataset was also collected but had huge problems with high mosaicity and the crystal died at the end of the dataset so not further data could be collected.

We also scanned and tested heavy atom derivative of Wo, Yb

During the final hour of our last shift we collected a quick native dataset 30-3.0 Å in C2 spacegroup. Redundancy 8.7 and Rsym 8.8 (28.6) with 99.9 % completeness.
This native dataset resulted in a succesful molecular replacement solution (CNS package) using a polyserine model with conserved residues from the *E.coli* R1 protein. Huge efforts to use molecular replacement in combination with experimental phases had previously been hampered by the C2 pseduo symmetry in the native P2(1) crystals.