ESRF	**Experiment title:** Crystallographic studies of enzymes involved in nucleotide metabolism	**Experiment number**: LS 1490
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Shifts: 9	**Local contact(s)**: Dr. Vivian Stojanoff	*Received at ESRF:*

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Report:

Dihydropyrimidine dehydrogenase (DPD) catalyzes the first and rate limiting step in pyrimidine breakdown, the NADPH-dependent reduction of pyrimidines to the corresponding 5,6-dihydropyrimidines. The complex enzyme is a dimer of identical subunits (1025 aa) and uses several cofactors, 1 FAD, 1 FMN and 4 [4Fe:4S]-clusters/subunit to control the electron flow from NADPH to the substrate. Besides the natural substrates uracil and thymine, DPD is also able to degrade 5-fluorouracil, a widely used anti-neoplastic drug, which decreases its efficiency as a therapeutic agent.

Multiple anomalous dispersion data for the native DPD were collected at three different wavelength around the iron edge, λ1 (peak) = 1.7392 Å to 3.0 Å, λ2 (inflection point) = 1.7416 Å to 3.0 Å, λ3 (remote) = 0.9184 Å to 1.9 Å. The crystal belongs to the space group P21 with cell dimensions a = 81.95 Å, b = 159.29 Å, c = 163.57 Å, β = 96.04°.
By using initial phase information obtained from a iridium derivative data set collected earlier we could easily locate all [4Fe:4S] clusters.

A NCS-averaged and solvent-flattened map calculated after refinement of the parameters for the [4Fe:4S] clusters using the ESRF-data was of excellent quality. Backbone tracing and

sequence assignment was straigthforward for most of the residues. Electron density for all cofactors could be identified, and the model of the enzyme could be built. Refinement of the structure is in progress.