ESRF	**Experiment title:** Single crystal X-ray diffraction experiments of eukaryotic transcription factor:DNA complexes	**Experiment number:** LS667
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Names and affiliations of applicants (* indicates experimentalists):

Christoph W. Müller, EMBL Grenoble Outstation
Patrick Cramer, EMBL Grenoble Outstation

Report:

Single X-ray diffraction experiments of two eukaryotic transcription factor:DNA complexes were carried out at BM14:

a) T domain:DNA complex

The T gene is the prototyp of a growing family of so-called T-box genes encoding transcriptional regulators identified in a variety of invertebrates and vertebrates including humans. The T-box encodes a DNA-binding domain of ca. 180 amino acid residues, the T domain.

The T domain from Xenopus laevis and zebrafish was cocrystallized with a 24-meric palindromic DNA target site. The two homologues crystallize in related space groups. (T Xenopus laevis, P21212 a=38 Å, b=114 Å, c=149 Å; T zebrafish, P21, a=38 Å, b=57 Å, c=149 Å. Both crystal forms diffract to about 2.0 Å resolution. Using synchrotron radiation at BM14 we were able to collect native data sets to 2.5 Å resolution from both homologues. The native data of the Xenopus laevis homologue together with iodine derivatives collected at a rotating anode and selenomethionine data collected at the swiss-

norwegian beamline (BMl) were combined in a m.i.r. analysis. The resulting electron density map permitted to build a model which was subsequently refined. The structure reveals that the T domain is bound as a dimer and it shows a novel type of specific DNA contact with a C-terminal deeply protruding into the minor groove of the DNA. A manuscript describing the structure has been accepted as a "Letter to Nature" [l]. The extension of the native data sets to higher resolution has so far been hampered by the radiation sensitivity of the crystals even at cryogenic temperature.

b) NF-κB P52:DNA complex

NF-κB is a transcription factor involved in inflammatory processes and response to infection in mammals. It is also deviated by HIV to control the transcription of its own genes. NF-κB P52 is a homologue of NF-κB P50, which had been solved previously.

By soaking in cryoprotectant a crystal form diffracting anisotropically to about 3.0 Å resolution was transformed into a highly diffracting crystal form (spacegroup P212121, a=44.2Å, b=121.0Å c=134.9Å). We were able to collect data from a NF-κB P52:DNA cocrystal to 2.1 Å resolution at BM14. Subsequently the structure was solved by molecular replacement and has been refined. A manuscript describing crystallization and data collection has been published [2]. The high resolution of this large protein:DNA complex (300 amino acid residues/monomer, bound as a dimer to DNA) permits the detailed discussion of protein:DNA recognition including the complex water structure in the protein:DNA interface. The manuscript describing the refined structure has been submitted to EMBO Journal [3].

[1] Müller, C.W. & Herrmann B. (1997).
Crystallographic structure of a T domain bound to DNA.
Nature (in press).

[2] Cramer, P. & Müller, C.W. (1997).
Engineering of Diffraction-quality Crystals of the NF-κB P52 Homodimer:DNA Complex. FEBS Letters 405, 373-377.

[3] Cramer, P., Larsen, C., Verdine, G. & Miiller, C.W. (1997).
Structure of the human NF-κB P52 Homodimer:DNA complex at 2.1 Å resolution. (submitted to EMBO Journal).