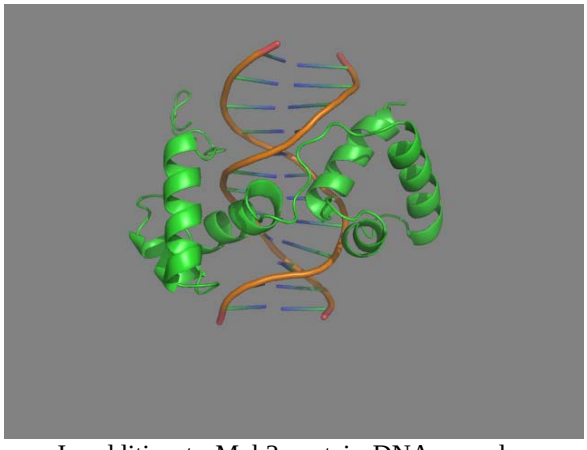
High Resolution Diffraction and MAD Experiment of Myb2-DNA Complexes from *Trichomonas vaginalis*

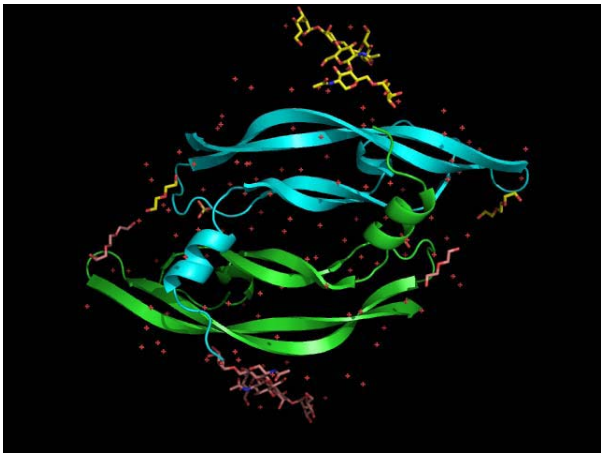
Imam Amiraslan, Yun-Wen Chen (陳韻雯), and Yen-Chywan Liaw (廖彥銓)

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Trichomonas vaginalis is a protozoan parasite. It causes the most common human nonviral sexually transmitted disease. The patients with trichomoniasis increase the transmission of the human immunodeficiency virus which is an imminent threat to public health. The parasite persistently inhabits only the human urogenital tract. Cytoadherence is crucial for *T. vaginalis* in infection and multiple surface adhesion proteins and lipophosphoglycans has been shown to involve. The iron supply modulate cytoadherence toward vaginal epithelial cells through transcriptional regulation of some of the adhesion protein *ap* genes, especially those in the *ap65* family. The *ap65-1* promoter contains eight closely spaced promoter elements, among which three Myb recognition elements (MRE), MRE-1/MRE-2r which overlap, and MRE-2f, are the binding sites for several Myb-likeDNA binding transcription factors. The recombinant Myb2 protein preferentially binds to MRE-2f to MRE-2r. Myb2 is involved in activation of growth-related and iron-inducible transcription of the *ap65-1* gene. We successfully collected Se-MAD data of Myb2-MRE-2r crystals diffract up to 2.0 A resolution at peak. We also collected native data sets of Myb2-MRE-2f complex crystals diffract up to 2.1 A. Currently we are trying to grow better crystals of Myb2-MRE-2r and try to analyze their difference and explain why Myb2 preferentially binds to MRE-2f to MRE-2r.



In addition to Myb2 protein-DNA complex we also carried out crystallographic studies of human VEGF and crystallographic studies of hyperthermophilic archaea dUTPase. Vascular endothelial growth factor (VEGF's) normal function is to create new blood vessels during embryonic development, new blood vessels after injury, and new vessels to bypass blocked vessels. VEGF is a potent mitogen in embryonic and somatic angiogenesis with a specificity for vascular endothelial cells. VEGF plays an important role in the pathogenesis of cancer, proliferative retinopathy, and rheumatoid arthritis. Antibodies against it have shown therapeutic potential as agents. VEGF binds to cell-surface receptors is of greater therapeutic interest. VEGF is a homodimer and exists in four different isoforms. the 110 N-terminal residues has shown to be the binding domain for KDR. Drugs such as bevacizumab can inhibit VEGF and control or slow those diseases. It is very important to have detail structural information of VEGF and its receptor plus its antibody. Currently very limited structural information is available. Previously, human VEGF (residues 8–109) was overexpressed in *Escherichia coli* inclusion bodies, and refolded to crystallize for structural studies. Our express by using S2 cell line to retain the glycosylation. Not only protein frame work but also glycosylation of this protein are clearly seen in the crystal structure. Detail of analysis is undergoing.



The ubiquitous enzyme dUTPase carry a crucial reaction to prevent uracil incorporation into DNA by catalyzing the cleavage of dUTP into UMP and pyrophosphate and strictly control the ratio of dUTP and dTTP in the cell. Since its special role in the cell, it become a target of cancer therapy. The potential intiative role of dUTPase antagonism in thymine-less cell death has lead the investigation into the enThe detail analysis is undergoing.