s8a.m8.o1 **Protein Crystallography and Structure Based Drug Design.** L.N. Johnson, M. E. M. Noble, J. A. Endicott, K. A. Watson & N. G. Oikonomakos*. *Laboratory of Molecular Biophysics, University of Oxford, Oxford OX1 3Q, UK &*Institute of Biological Research, The National Hellenic Research Foundation, Athens 11635, Greece.*
Keywords: drug design.

The notion that drugs might target a specific receptor and thereby effect a limited number of biological processes which relate to disease has had a profound influence on drug design. The revolution in biology, especially genomic sequencing, over the last decade has given a greatly increased number of molecular targets that give rise to radical new opportunities for drug design. Once a target is identified and its structure determined, the task of designing a specific inhibitor would appear to be simple. Yet the number of products that have reached the market based on this philosophy are relatively few compared with those achieved by more conventional screening of large numbers of compounds from data bases. Structure based drug design successes include the treatment of glaucoma with a carbonic anhydrase inhibitor, the treatment of AIDS with a combination of HIV protease and reverse transcriptase inhibitors, and the alleviation of influenza with an flu viral neuraminidase inhibitor. The development of a good inhibitor to a therapeutic agent requires the specialist expertise of pharmaceutical companies. Protein crystallography can contribute information on the specificity and interactions that distinguish the binding site and can reveal unexpected binding sites.

The lecture will review the current status of protein crystallography in structure based drug design and will summarise structural results our laboratories. Recent work has focused on inhibition of the cell cycle regulatory kinase, CDK2/cyclin A, both by natural product inhibitors [1, 2] and by designed agents with the ultimate aim of finding an agent for use in the control of unregulated cell proliferation and allosteric inhibitors of glycogen phosphorylase [3-5] that prevent unwanted glycogenolysis under high glucose conditions and may be relevant to the control of diabetes. Indeed a compound that targets both enzymes, CDK2 and phosphorylase, could have an added benefit in starving cancer cells of glucose at the same time disrupting the cell cycle and sending cells into apoptosis.

[1] Lawrie, A.M., *et al.* "Protein kinase inhibition by staurosporine: details of the molecular interaction determined by X-ray crystallographic analysis of a CDK2-staurosporine complex." *Nature Structural Biology* , (1997), 4: 796-801 .
[2] Hoessel, R., *et al.* "Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases." *Nature Cell Bioilogy* , (1999), 1: 60-67 .
[3] Gregoriou, M., *et al.* "The structure of a glycogen phosphorylase glucopyranose spirohydantoin complex at 1.8 Å resolution and 100 K: the role of the water structure and its contribution to binding." *Protein Science* , (1998), 7: 915-927 .
[4] Zographos, S.E., *et al.* "A novel inhibitor of glycogen phosphorylase." *Structure* , (1997), 5: 1413-1425 .
[5] Oikonomakos, N.G., Skamnaki, V.T., Tsitsanou, K.E., Gavaloas, N.G. & Johnson, L.N. "A new allosteric site on glycogen phosphorylase b as a target for drug interactions." *Structure* , (2000), In press: .

s8a.m8.o2 **Dihydropyrimidine dehydrogenase – a target for improved cancer therapy.** G. Schneider, *Department of Medical Biochemistry & Biophysics, Karolinska Institutet, Stockholm, Sweden*
Keywords: drug design.

The degradation of the pyrimidines uracil and thymine occurs by a three-step pathway resulting in the products β-alanine and β-aminoisobutyrate, respectively. The same pathway is used to degrade some chemotherapeutic agents, for example 5-fluorouracil. This drug is one of the most commonly prescribed anti-cancer agents and inhibits thymidylate synthase, an enzyme in nucleotide biosynthesis. The efficient degradation of 5-fluorouracil by the catabolic pathway reduces its efficiency in chemotherapy and requires the application of extremely high doses. In addition, the breakdown of 5-fluorouracil leads to fluorinated products causing multiple drug side effects in the nervous system. Any inhibition of the catabolic pyrimidine pathway could increase the clinical potential of 5-fluorouracil and related compounds by increasing their effectiveness, resulting in the application of lower doses and possibly diminished side effects. The rate limiting enzyme of this pathway, and thus the most obvious target for inhibition, is dihydropyrimidine dehydrogenase. Mammalian dihydropyrimidine dehydrogenases are large enzymes, they consist of homodimers of a polypeptide chain comprising about 1025 amino acids.

We have determined the three-dimensional structure of this enzyme by protein crystallography using multi-wavelength anomalous diffraction, based on the four iron-sulfur clusters in the enzyme subunit. X-ray data were collected at beamline BM14 at the ESRF, Grenoble, France. The structure determination reveals a highly modular composition of the enzyme subunit, consisting of five domains, two structurally different domains binding the four Fe-S clusters, one α/β barrel domain binding the FMN cofactor and two domains binding FAD and NADPH, respectively. The active site is located at the carboxy-end of the β-strands of the α/β barrel domain. The 3D structure reveals an extended pathway for electron transfer from NADPH to the substrate, involving seven cofactors which makes this enzyme one of the most complex soluble proteins catalyzing electron transfer. The architecture of the substrate/inhibitor binding sites and prospects for structure-assisted drug design will be discussed.